

Application of Selected Ion Flow Tube-Mass Spectrometry to the Characterization of Monofloral New Zealand Honeys

Vaughan Langford,[†] John Gray,[†] Bob Foulkes,[‡] Peter Bray,[§] and Murray J. McEwan^{*,†,||}

[†]Syft Technologies Ltd., Christchurch, New Zealand

[‡]Quintessence Developments Ltd., Christchurch, New Zealand

[§]Airborne Honey Ltd., Christchurch, New Zealand

^{||}Department of Chemistry, University of Canterbury, Christchurch, New Zealand

S Supporting Information

ABSTRACT: Honeys have a range of physicochemical and organoleptic properties, depending on the nectar source. Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) is an emerging technology that quantifies volatile organic compounds (VOCs) to low concentrations (usually parts-per-trillion (ppt) levels) and is here applied to monitor the aromas in the headspace of different New Zealand monofloral honeys. Honey aromas arise from VOCs in the honeys that differ according to the flower type from which they were derived. In this exploratory study, the headspaces of nine monofloral New Zealand honeys (beech honeydew, clover, kamahi, manuka, rata, rewarewa, tawari, thyme, and vipers bugloss) were analyzed using SIFT-MS without sample preparation. The purpose of the investigation was to identify the major volatiles in each of the honeys and to test the feasibility of using SIFT-MS to distinguish between New Zealand monofloral honeys. In the nine monofloral honeys sampled, a clear distinction was observed between them based on their aroma signatures.

KEYWORDS: honey, manuka honey, SIFT-MS, honey aroma

■ INTRODUCTION

New Zealand (NZ) is a small, southwest-Pacific nation that has a diverse native and introduced flora. A number of the native and introduced species produce nectar that bees gather, and regional concentrations together with unique flowering periods allow monofloral honeys to be collected, processed, and marketed. The most common of these are clover, kamahi, manuka, rata, rewarewa, tawari, thyme, and vipers bugloss honeys and beech honeydew (where the NZ beech species differ from the northern hemisphere ones).

Manuka is the most prized of the NZ honeys both locally and internationally because of health benefits offered by high antibacterial and significant antioxidant activities.^{1–10} Because manuka honey offers higher profits, a key issue for the consumer is having confidence in the purity of the product they are purchasing. Traditionally, honeys have been determined as monofloral by using a combined approach of physicochemical and organoleptic testing, and pollen identification and counts to determine that they are wholly or mainly derived from a particular flower type.¹¹ There are situations when these procedures cannot easily detect dilution of the honey with other honeys that have, for example, similar colors, weak flavors, or low pollen representations. We note that probably no honey produced by free flying bees is truly monofloral and that the term monofloral is used to describe honeys in which the majority part of the nectar or honeydew is derived from a single plant species.¹²

A possible alternative to traditional methods of authentication lies in analysis of the aroma of the honey, that is, analysis of the volatile organic compounds (VOCs) in the headspace of the honeys that impart their unique aroma. Many studies have

been undertaken on specifying VOCs that are most closely associated with a specific honey.^{13–20} However, few studies have addressed the range of VOCs present in New Zealand honeys, with most of the emphasis focusing on volatile fatty acids and phenolics^{8,21–26} or specific studies that might be indicative of the unique properties discovered in the honey.^{24–26}

In this work, we have applied Selected Ion Flow Tube Mass Spectrometry (SIFT-MS), an emerging direct mass spectrometry technique, to the analysis of honeys for the first time. SIFT-MS analyzes gas samples for VOCs and quantifies these compounds in real-time at very low concentrations (usually to parts-per-trillion (ppt) levels), even at high humidity. This study of nine monofloral NZ honeys represents an exploratory first stage in assessing the probability of a rapid SIFT-MS test for honey purity by means of the aroma compounds in the headspace of honey.

■ MATERIALS AND METHODS

Honey Laboratory Measurements. Nine monofloral honeys from Airborne Honey, a leading New Zealand honey processor, were used in this study. The purpose of the measurements performed in the honey laboratory was to obtain a valid quality control measure of the honey from the suppliers. Table 1 provides a summary of the honeys and their properties, which were determined in the Airborne Honey laboratory using standard methods. A pollen analysis of the honey provides one basis for identifying the honey in terms of locality and floral source. Pollen analysis included count and total pollen grains per 10 g;

Received: February 16, 2012

Revised: June 11, 2012

Accepted: June 16, 2012

Published: June 28, 2012

Table 1. Properties of the New Zealand Monofloral Honey Analyzed in This Work

honey type	code used	batch no.	pollen percentage	color ^a	moisture (%)	HMF (mg kg ⁻¹)	fructose (%)	glucose (%)
beech honeydew	BH	082716	N/A ^b	91	15.3	1.38	29.3	14.4
clover	Cl	080752	84.7	30	17.0	4.21	37.7	32.3
kamahi	Ka	082632	66	45	17.4	0.98	34.7	30.7
manuka	Ma	083721	85.7	86	17.1	8.48	37.6	28.9
rata	Ra	083951	52.3	28	17.0	5.86	38.5	34.0
rewarewa	Re	071631	3.3	96	16.5	0.96	35.3	29.1
tawari	Ta	084051	6	42	17.3	3.98	38.9	30.2
thyme	Th	070923	NM ^c	92	16.8	2.15	37.9	32.2
vipers bugloss	VB	081011	65.2	28	16.2	8.59	37.3	32.9

^aColor units are mm on the Pfund scale in which water is zero and the maximum dark reading is 140. ^bBeech honeydew is not a nectar source honey. ^cNot measured.

Table 2. Summary of Selected Volatile Compounds Found in Various Honey from Different Countries of Origin

compd name	honey type(s) and origin(s)	refs
formic acid	manuka and thyme (NZ)	29
acetic acid	haze (Japan)	30
isobutyric acid	Caju and Marmeleiro (Brazil)	31
2-methylpropanoic acid	various	32
isovaleric acid (3-methylbutanoic acid)	Caju and Marmeleiro (Brazil); cotton (Greece); haze (Japan)	30, 31, 33
2 and 3-methylbutanoic acid	cotton (Greece); various	32, 33
octanoic acid	haze (Japan); manuka and kanuka (NZ); various (Lithuania); chestnut	23, 30, 34, 35
2-ethylhexanoic acid	<i>Thymelaea hirsuta</i> (Palestine)	36
nonanoic acid	haze (Japan); thyme (Greece); <i>Thymelaea hirsuta</i> (Palestine); various	30, 35–37
decanoic acid	thyme (Greece); chestnut	35, 37
benzoic acid	Caju and Marmeleiro (Brazil); cotton (Greece); clover (NZ); manuka and kanuka (NZ)	23, 31, 33
4-methoxybenzoic acid	manuka (NZ)	21
<i>m</i> -toluic acid	cotton (Greece)	33
phenylacetic acid	Caju (Brazil); cotton (Greece); clover (NZ); haze (Japan); manuka and kanuka (NZ)	23, 30, 31, 33
phenylpropanoic acid	cotton (Greece)	33
α -hydroxybenzene propanoic acid	thyme	35
<i>p</i> -anisic acid	cotton (Greece)	33
<i>m</i> -salicylic acid	cotton (Greece)	33
acetone	various	32
hydroxyacetone	various	32
2-butanone	various	32
3-hydroxy-2-butanone	various	32, 35
3-methyl-2-butanone	various	32
3-hydroxy-2-pentanone	various	32
5-hepten-2-one-6-methyl	various	32
2-cyclohexene-1-one	various	32
2-cyclohexen-1-one-3,5,5-trimethyl	various	32
2,2,6-trimethylcyclohexanone	various	32
acetophenone	thyme (Greece; NZ); manuka (NZ); various (European), chestnut	29, 32, 35, 37, 38
3-aminoacetophenone	various	32, 35
<i>p</i> -methylacetophenone	lime tree	35
2'-methoxyacetophenone	clover (NZ)	23
4-methoxyacetophenone	manuka (NZ)	21
2,4-dimethylacetophenone	haze (Japan)	30
3,4-dimethylacetophenone	haze (Japan)	30
3-hydroxy-4-phenyl-2-butanone	thyme (Greece)	37
3-hydroxy-1-phenyl-2-butanone	thyme (Greece)	37
1,3-diphenyl-2-propanone	<i>Thymus capitalus</i> (Palestine)	36
isophorone	thyme (Greece)	37, 38
4-oxoisophorone	various (European); eucalyptus	32, 35, 38
β -damascenone	lime tree; dandelion	35
1,5-bis(1,1-dimethylethyl)-3,3-dimethylbicyclo[3.1.0]hexan-2-one	haze (Japan)	30
9-methyl-2-decalone	manuka (NZ)	21
2,3-pentanedione	various	32

Table 2. continued

compd name	honey type(s) and origin(s)	refs
cyclopentadione	various	32
methylbutanals	various	32
pentanal	various	32
hexanal	various (European); clover (Canada); various	32, 38, 39
heptanal	various (European)	38
octanal	various (European)	32, 34, 38
nonanal	cotton (Greece); thyme (Greece); <i>Thymelaea hirsuta</i> (Palestine); various	32, 33, 35–38
decanal	cotton (Greece); <i>Thymelaea hirsuta</i> (Palestine); various (Europe and Lithuania)	32–34, 36, 38
dodecanal	<i>Thymelaea hirsuta</i> (Palestine)	36
(E)-cinnamaldehyde	cotton (Greece); chestnut	33, 35
benzaldehyde	clover (Canada); cotton (Greece); haze (Japan); thyme (Greece; NZ); <i>Thymelaea hirsuta</i> (Palestine); manuka (NZ); various	21, 29, 30, 32, 35–38
phenylacetaldehyde	clover (Canada); cotton (Greece); thyme (Greece); manuka (NZ); various	21, 27, 32, 33, 35, 37
4-methoxybenzaldehyde (p-anisaldehyde)	haze (Japan); chestnut	30, 35
3,4-dimethoxybenzaldehyde	<i>Thymus capitalus</i> (Palestine); chestnut	35, 36
3,4,5-trimethoxybenzaldehyde	<i>Thymus capitalus</i> (Palestine)	35, 36
vanillin	Marmeleiro (Brazil); <i>Thymus capitalus</i> (Palestine)	31, 36
lilac aldehydes (I, II, III)	various (European)	34, 35, 38
bicyclo[3.1.1]-hept-2-ene-2-carboxaldehyde	manuka (NZ)	29
ethanol	various	32, 35
2-phenol ethanol	manuka (NZ)	21
2-propanol	haze (Japan); various	30, 32
2-methyl-1-propanol	various	32
1-butanol	various	32
methylbutanols	various	32
methyl-2-butenol	various	32
2,3-butanediol	haze (Japan)	30
2-methyl-1-butanol	manuka and thyme (NZ); eucalyptus	29, 35
3-methyl-3-buten-1-ol	manuka and thyme (NZ); eucalyptus; chestnut	29, 35
1- and 2-pentanol	various	32
1-penten-3-ol	various	32
1-pentanol-4-methyl	various	32
hexan-1-ol	clover (Canada); <i>Thymelaea hirsuta</i> (Palestine); various (European)	32, 36, 38
3-hexen-1-ol	various	32
5-methyl-1-hexanol	various	32
2-butoxyethanol	various	32
heptan-1-ol	various (European)	38
octanol	various (European)	38
1-octen-3-ol	various	32
hotrienol (2,6-dimethyl-1,3,7-octatrien-6-ol)	haze (Japan); various (Lithuania)	30, 32, 36
bicyclo-2,2,2-octan-1-ol-4-methyl	various	32
nonan-1-ol	various (European)	35, 38
decan-1-ol	various (European)	38
(E)-cinnamyl alcohol	cotton (Greece)	33
phenol	clover (Canada); eucalyptus	35, 39
trimethylphenol	eucalyptus	35
benzenemethanol (benzyl alcohol)	Caju (Brazil); clover (Canada); cotton (Greece); haze (Japan); <i>Thymelaea hirsuta</i> (Palestine); various	30–33, 35, 36, 39
2-phenylethanol (phenylethyl alcohol)	Caju and Marmeleiro (Brazil); clover (Canada); cotton (Greece); haze (Japan); thyme (Greece); <i>Thymelaea hirsuta</i> (Palestine)	30, 31, 33, 35, 38
methylbenzyl alcohol	various	32, 35
benzenepropanol	cotton (Greece); <i>Thymelaea hirsuta</i> (Palestine)	33, 36
phenylethyl alcohol	various	32
1-phenyl-2-butanol	haze (Japan)	30
1-phenyl-1-pentanol	haze (Japan)	30
2,6-bis(1,1-dimethylethyl)-4-methylphenol	haze (Japan)	30
2-allyl-4-methylphenol	various	32
2-methoxyphenol	Caju (Brazil)	31

Table 2. continued

compd name	honey type(s) and origin(s)	refs
4-methoxyphenol	<i>Thymelaea hirsuta</i> (Palestine)	36
4-methoxybenzenemethanol	cotton (Greece); haze (Japan)	30, 33
4-methoxybenzeneethanol	thyme; lime tree	35
3-hydroxybenzeneethanol	haze (Japan)	30
4-hydroxybenzeneethanol	cotton (Greece)	33
1,4-dihydroxybenzene	manuka (NZ)	21
3,5-dihydroxytoluene	<i>Tolpis virgata</i> (Palestine)	36
lilac alcohols	thyme; citrus	35
ethyl formate	manuka (NZ)	21
ethyl acetate	haze (Japan)	30, 32
allylphenyl acetate	manuka (NZ)	21
ethenylphenyl acetate	dandelion	35
ethyl-2-hydroxypropanoate	various	32
butyl butanoate	Caju (Brazil)	31
methyl pentanoate	manuka (NZ)	21
3-hexenyl formate	various	32
(<i>E</i>)-3-hexenyl hexanoate	haze (Japan)	30
methyl benzoate	manuka (NZ)	21
methyl-3,5-dimethoxy benzoate	manuka (NZ)	21
methyl anthranilate	thyme; citrus	35
ethyl benzoate	various	32
isopropyl benzoate	manuka (NZ)	21
ethyl heptanoate	various (European)	38
ethyl octanoate	various (European)	38
ethyl nonanoate	various (European)	38
ethyl decanoate	various (European)	38
2-methylfuran	clover (Canada)	32, 39
2-ethylfuran	various	32
acetylfuran	clover (Canada); various	32, 39
2-carbomethoxyfuran	manuka (NZ)	21
2,3-dihydro-4-methylfuran	various	32, 35
2-methyldihydrofuranone	various	32
2-methyltetrahydrofuran-3-one	manuka (NZ)	21
5-ethenyl-5-methyl-2(3 <i>H</i>)-furanone (lavender lactone)	haze (Japan)	30
5-hydroxymethylfurfural (HMF)*	haze (Japan); <i>Thymelaea hirsuta</i> (Palestine); manuka (NZ); various	30, 35, 36
furfural	clover (Canada); cotton (Greece); various (European)	32–34, 38, 39
5-methylfuraldehyde	clover (Canada); dandelion	32, 35, 39
furfural alcohol	clover (Canada)	32, 39
furfural <i>n</i> -butyrate	citrus; dandelion	35
furyl hydroxymethyl ketone	haze (Japan)	30
3,5-dihydroxy-2-methyl-4 <i>H</i> -pyran-4-one (hydroxymaltol)	haze (Japan)	30
gamma-butyrolactone	various	32
delta-octalactone	Caju and Marmeleiro (Brazil)	31
gamma-decalactone	Caju and Marmeleiro (Brazil)	31
coumarin	clover (Canada)	39
linalool	haze (Japan); Marmeleiro (Brazil); <i>Thymelaea hirsuta</i> (Palestine); various (Lithuania)	30–32, 34–36
linalool oxide (various isomers)	haze (Japan); Marmeleiro (Brazil); chestnut; thyme	30–32, 35
camphor	clover (Canada)	39
isoborneol	lime tree	35
<i>cis-p</i> -menth-8-ene-1,2-diol	haze (Japan)	30
menthol	Caju and Marmeleiro (Brazil)	31
<i>p</i> -cymen-8-ol	lime tree	35
carvacrol	thyme (Greece); lime tree	34, 35, 37
nerolidol	citrus	35

Table 2. continued

compd name	honey type(s) and origin(s)	refs
terpinen-7-al	lime tree	35
furfural mercaptan	Caju (Brazil)	31
dimethyl sulfide	manuka and thyme (NZ); various (Lithuania)	29, 34
dimethyl disulfide	various	32
dimethyl trisulfide	various (European)	38
<i>m</i> -xylene	Clover (Canada)	39
<i>p</i> -cymene	various (European)	38
(3-methylbutyl)benzene	<i>Thymus capitalus</i> (Palestine)	36
4-methoxypropylbenzene	manuka (NZ)	21
alpha- and beta-pinene	clover (Canada)	39
limonene	cotton (Greece)	33
<i>trans</i> -caryophyllene	clover (Canada)	39
octane	clover (Canada)	39
tridecane	haze (Japan); <i>Tolpis virgata</i> (Palestine)	30, 36
tetradecane	cotton (Greece)	33
2-methylpropanenitrile	various	32
2-methylbutanenitrile	various (Lithuania)	34
pentanenitrile	various (Lithuania); dandelion	34, 35
C6 nitrile	dandelion	35
benzyl nitrile	thyme (Greece); various (Lithuania)	34, 37
geranyl nitrile	cotton (Greece)	33
phenylacetone nitrile	thyme (Greece)	37
chloroform	various	32

the color analysis was monitored using a Kohler Pfund grader; and moisture was measured with a refractometer calibrated for honey. The sugars and hydroxymethyl furfural (HMF) were monitored using HPLC. The separation of sugars was performed on a normal phase NH₂ column using a complex quaternary solvent gradient profile at elevated temperature. Detection was by both refractive index detector (RID) and evaporative light scattering detector (ELSD) in a 50:50 split-flow parallel configuration. The ELSD detection was at 45 °C and 3.5 bar pressure using air as the nebulizer gas. HMF analysis was achieved on a C18 column using CH₃OH/H₂O eluent with detection at 284 nm. The organic compound profile analysis was obtained by gradient analysis on the same C18 column using a CH₃OH/H₂O eluent with analysis by a photodiode array detector (PDAD) at various UV wavelengths.

HMF is monitored as it is a key indicator of the storage and handling history of the honey. Historically, much of the small scale honey processing was not well controlled and sometimes was destructive to the honey quality. Poor quality or highly processed honey gets heated excessively and darkens with a corresponding increase in HMF levels. International standards specify a limit to the HMF content for this reason, and the HMF levels of the selected honeys were well under these limits.

Chemicals. All solvents for the HPLC measurements were obtained from commercial sources and were of HPLC grade. HMF and sugars used as internal standards were all Analar grade from commercial sources. The reference compounds for the identification of odorants were purchased from commercial sources as follows: 3-methyl-1-butanol, hexanol, nonanol, and phenylacetaldehyde (Aldridge); 3-methylbutanoic acid (Sigma Aldridge); coumarin (Sigma); linalool (Fluka); dimethylsulfide (Acros); methanol, benzoic acid, phenylacetic acid, benzaldehyde, and 1-hexanal (BDH); 2-methylfuran, acetone, phenylmethanol, and 2-phenylethanol (Merck); and acetaldehyde and acetophenone (Riedel-de Haen).

SIFT-MS Measurements. Honey samples were stored at room temperature prior to analysis. Five grams of honey were placed in 500-mL Schott bottles capped with pierceable septa. These honey samples used for analysis came from extracting small samples of each monofloral honey after they had been well mixed in a large holding tank. The tank contained a specific monofloral honey contributed from multiple apiaries and collected over a wide geographic region. These samples are therefore representative of the honey types they represent as the quantities from which the samples came ranged from 660 kg for beech honeydew to 6,212 kg for manuka honey produced in six different apiaries. For mass scan analysis, samples were prepared in duplicate, and each was analyzed three times to give a total of six analyses for each honey. For Selected Ion Mode analysis, five replicate samples were prepared for each honey. Samples were incubated at 30 °C for approximately one hour before being analyzed.

SIFT-MS analysis. SIFT-MS is an analytical technique that uses chemical ionization reactions coupled with mass spectrometric detection to rapidly quantify targeted VOCs.^{27,28} VOCs are identified and quantified in real time from whole-gas samples based on the known rate coefficients for reaction of the chemical ionization species (so-called reagent ions) with the target analytes. The method has been discussed in some detail elsewhere,^{27,28} and the details and methodology will not be repeated here. It is sufficient to say that the instrument can be used in two modes of analysis: a mass scan (MS) mode where a mass spectrum is recorded of volatiles in the headspace and a selected ion mode (SIM) where the instrument samples the ion products of known volatiles.

RESULTS AND DISCUSSION

Known Volatiles in Honey Headspace. A large number of compounds have been identified in honey aromas using a variety of techniques. Some of the more volatile compounds that have been reported in the literature are summarized in Table 2 with the flower type from which the honey is derived.

Table 3. Reagent and Product Ions Used to Quantify Target Compounds (Table 4) and Potential Interferents^a

volatile honey compds	reagent and product ions used in the method			comments	potential interferents (at best performing masses)
	H ₃ O ⁺	NO ⁺	O ₂ ⁺		
methylbutanoic acids	103	85, 132		both NO ⁺ ions products at 85 and 132 were used	
benzoic acid	123	122		both H ₃ O ⁺ and NO ⁺	
phenylacetic acid	91	91, 136		both H ₃ O ⁺ and NO ⁺	
2-methylfuran		82	82	NO ⁺ only	
furfural	97	95, 96, 126	96	NO ⁺ only/96	
HMF	127	126	126	H ₃ O ⁺ only	
methanol	33 (+W)	62		H ₃ O ⁺ only	
ethanol	47 (+W)	45 (+W)		NO ⁺ only	
3-methyl-1-butanol	71	87	59	both H ₃ O ⁺ and NO ⁺	
1-hexanol	85	101		NO ⁺ only	
phenylmethanol	91	107, 108	107, 108	NO ⁺ only/108	
2-phenylethanol	105	122	92	NO ⁺ only	
acetaldehyde	45 (+W)	43 (+W)		usually H ₃ O ⁺	
hexanal	101	99		NO ⁺ only	
nonanal	143	141		NO ⁺ only	
benzaldehyde	107	105	105, 106	O ₂ ⁺ only/106; sometimes NO ⁺	
phenylacetaldehyde	121	120, 150	91, 92	NO ⁺ /120 for all but one honey (NO ⁺ /150)	acetophenone (NO ⁺ /150)
acetone	59 (+W)	88		H ₃ O ⁺ only	
acetophenone	121	150	105, 120	NO ⁺ /150 for all but one honey (O ₂ ⁺ /120)	phenylacetaldehyde (NO ⁺ /150)
dimethyl sulfide	63	62	62	NO ⁺ only	
linalool	81, 137	136	80, 93, 137	both H ₃ O ⁺ and NO ⁺	monoterpenes and various terpene oxygenates
coumarin	147	146		usually H ₃ O ⁺	

^aIons that perform best are indicated in bold. +W = water clusters.

Table 4. Concentrations (in µg/L) of Compounds in the Headspaces of Nine New Zealand Honeys at 30 °C, As Measured Using a Syft Technologies Voice200 SIFT-MS Instrument in Selected Ion Mode

compds	beech honeydew		clover		Kamahi		manuka		Rata		Rewarewa		Tawari		thyme		Vipers Bugloss	
	mean	SD ^a	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
methylbutanoic acids	.37	0.08	.45	0.04	.75	0.19	1.13	0.09	.12	0.02	.59	0.07	.54	0.67	.084	0.009	1.50	0.035
benzoic acid	.094	0.008	.084	0.005	.124	0.023	.089	0.004	.049	0.005	.043	0.005	.084	0.009	.040	0.014	.064	0.009
phenylacetic acid	.766	0.120	.766	0.019	.602	0.125	.657	0.071	.333	0.060	.372	0.060	.766	0.104	.170	0.036	.443	0.041
2-methylfuran	.119	0.001	.178	0.028	.145	0.023	.528	0.082	.020	0.004	.033	0.005	.132	0.026	.016	0.004	.271	0.030
furfural	.085	0.004	.100	0.012	.116	0.025	.120	0.011	.037	0.010	.026	0.003	.147	0.016	.050	0.010	.147	0.017
HMF	1.06	0.506	.375	0.050	.375	0.046	.608	0.152	.091	0.012	.086	0.002	.213	0.023	.112	0.006	.137	0.010
methanol	2.70	0.21	2.19	0.10	2.32	0.33	4.12	0.27	1.67	0.26	1.25	0.17	6.43	0.76	1.16	0.19	1.93	0.22
ethanol	24.0	2.40	15.0	1.11	48.1	5.10	14.4	1.33	11.8	1.85	16.3	2.22	24.0	3.88	3.33	0.72	20.3	2.40
3-methyl-1-butanol	.078	0.011	.074	0.006	.960	0.149	.103	0.110	.043	0.007	.461	0.004	.074	0.01	.021	0.005	.056	0.008
1-hexanol	.037	0.002	.028	0.019	.074	0.013	.058	0.07	.018	0.003	.024	0.003	.053	0.011	.012	0.003	.029	0.008
phenylmethanol	.122	0.011	.139	0.007	.139	0.025	.135	0.009	.100	0.019	.091	0.013	.135	0.015	.041	0.006	.074	0.006
2-phenylethanol	.113	0.010	.103	0.006	.138	0.022	.108	0.005	.059	0.005	.069	0.007	.098	0.014	.059	0.014	.093	0.008
acetaldehyde	.443	0.055	.885	0.071	.761	0.116	1.13	0.158	.336	0.060	.354	0.064	.885	0.024	.078	0.014	.212	0.037
hexanal	.109	0.006	.085	0.004	.109	0.013	.109	0.010	.044	0.007	.052	0.004	.085	0.010	.037	0.010	.077	0.006
nonanal	.114	0.009	.120	0.009	.120	0.026	.166	0.015	.036	0.007	.031	0.003	.074	0.015	.035	0.004	.063	0.004
benzaldehyde	.294	0.051	.243	0.008	.392	0.123	.285	0.029	.137	0.026	.137	0.018	.324	0.060	.055	0.011	.209	0.035
phenylacetaldehyde	.217	0.015	.198	0.010	.198	0.039	.174	0.023	.164	0.028	.072	0.002	.174	0.027	.184	0.028	.188	0.012
acetone	3.26	0.51	3.96	0.72	2.33	0.77	2.33	0.74	.420	0.02	1.35	0.02	2.80	0.02	2.24	0.01	2.80	0.02
acetophenone	.222	0.02	.178	0.01	.272	0.06	.307	0.02	.258	0.05	.154	0.03	.466	0.08	.109	0.03	.198	0.02
dimethyl sulfide	.245	0.02	6.240	0.82	.475	0.15	2.15	0.32	.140	0.03	.080	0.01	1.30	0.40	.040	0.01	.135	0.02
linalool	.509	0.05	.620	0.05	.577	0.130	.502	0.03	.192	0.08	.341	0.04	.682	0.105	.136	0.01	.310	0.04
coumarin	.818	0.03	.684	0.05	.900	0.19	.858	0.04	.214	0.04	.295	0.02	.818	0.15	.228	0.02	.469	0.03

^aSD refers to the standard deviation of the mean.

The emphasis in Table 2 is a compilation of compounds that might be found in a rapid sampling of honey headspace without

resorting to preconcentration methods such as SPME or solvent extraction. For this reason, some of the less volatile compounds

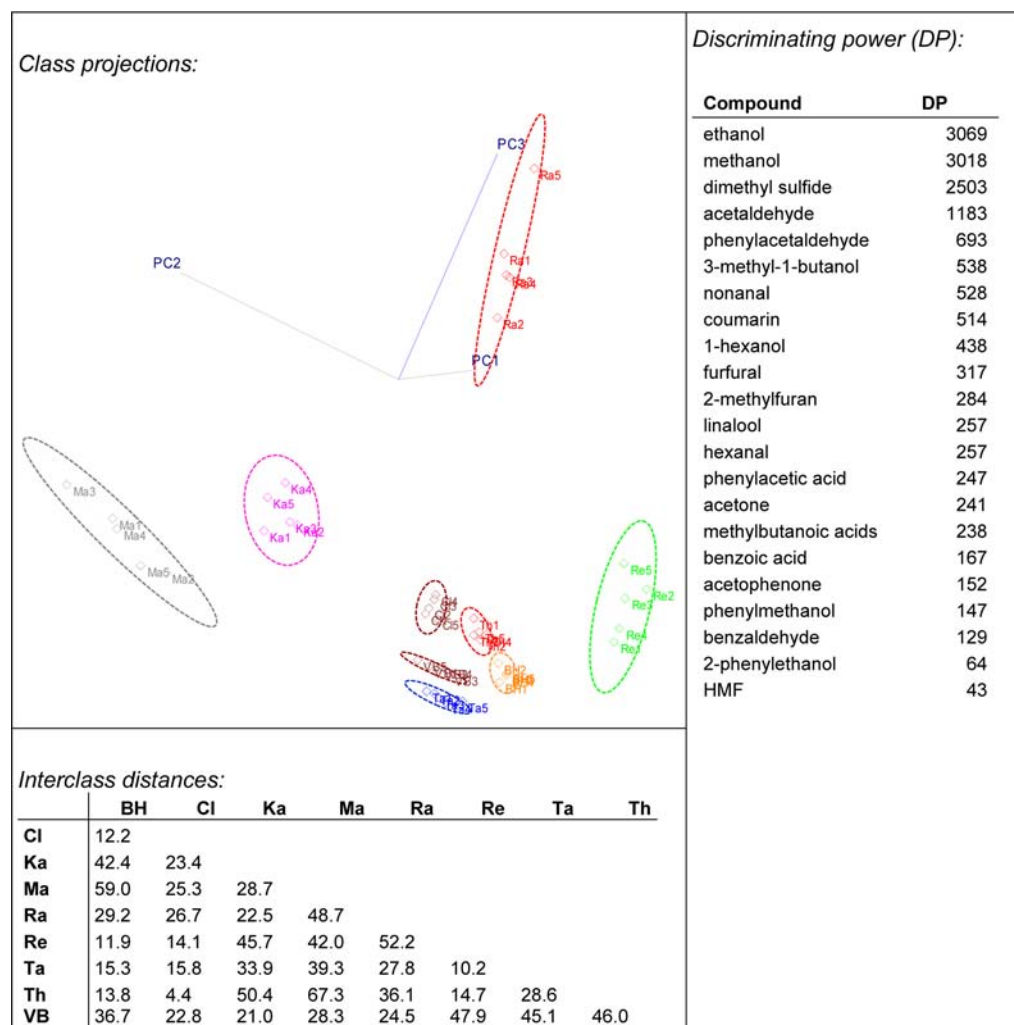


Figure 1. SIMCA multivariate analysis of SIFT-MS SIM data from New Zealand monofloral honeys (see Table 2). All compounds in the method are shown. The key to the honey types shown in the interclass distances is given in Table 1. See the text for further details.

such as dicarboxylic acids, diols, and larger carboxylic acids, aldehydes, esters, and ketones have been excluded. Instrumental analyses for VOCs have traditionally involved gas chromatography–mass spectrometry (GC-MS), but the differing extraction methods (from solvent extraction to solid-phase microextraction (SPME)) potentially means that quite different VOCs are emphasized.^{13,19,20} The list of volatile compounds shown in Table 2 was also augmented by a thermal desorption gas chromatograph investigation in our laboratory of New Zealand honeys from the list of honeys investigated in this work in Table 2. These results are also included in Table 2 and are shown as ref 29.

Mass Scan Analysis. Each reagent ion (H_3O^+ , NO^+ , and O_2^+) generates a mass scan from the honey headspace sample that arises from the ion products of the reaction of the reagent ion with the aroma volatiles of the honey. One such mass scan is shown in the Supporting Information where the difference between white clover and manuka honey is presented for the reagent ion NO^+ . Similar mass spectra are found for the H_3O^+ and O_2^+ reagent ions. In order to identify what the volatiles are in complex mixtures of many volatiles, it is usually necessary to have some prior knowledge of the volatiles that are likely to be present. Not a lot is known about the more volatile compounds present

in New Zealand honeys, although this knowledge has been extended by the data in Table 2. It is possible, however, to do a statistical analysis from mass scan data based on differences in ion abundance alone. In fact, a useful preliminary discriminatory fingerprint of each of the nine honeys is possible based on this “black box” approach for the honey samples supplied.

A possible weakness in the mass scan approach, however, is the potential variability of compounds like acetaldehyde, ethanol, and methanol that often provide good discrimination since they are fairly ubiquitous and volatile. Because they are at relatively high concentrations in the headspace, a number of their ^{13}C isotope and dimer and trimer peaks also register as important discriminators using the “black box” approach. For example, the reagent ion H_3O^+ reacts with ethanol to form product ions $\text{C}_2\text{H}_5\text{OH}_2^+$ at m/z 47, $\text{C}_2\text{H}_5\text{OH}_2^+(\text{H}_2\text{O})$ at m/z 65 and $\text{C}_2\text{H}_5\text{OH}_2^+(\text{C}_2\text{H}_5\text{OH})$ at m/z 93. In the “black box” approach, contributions from $\text{C}_2\text{H}_5\text{OH}$ are therefore counted more than once. Hence a better approach is to target actual compounds in a SIM analysis and build a discrimination model based on these. The remainder of this work focuses on identification of the honeys based on specific known volatile compounds that were quantified from the headspace using the SIM method.

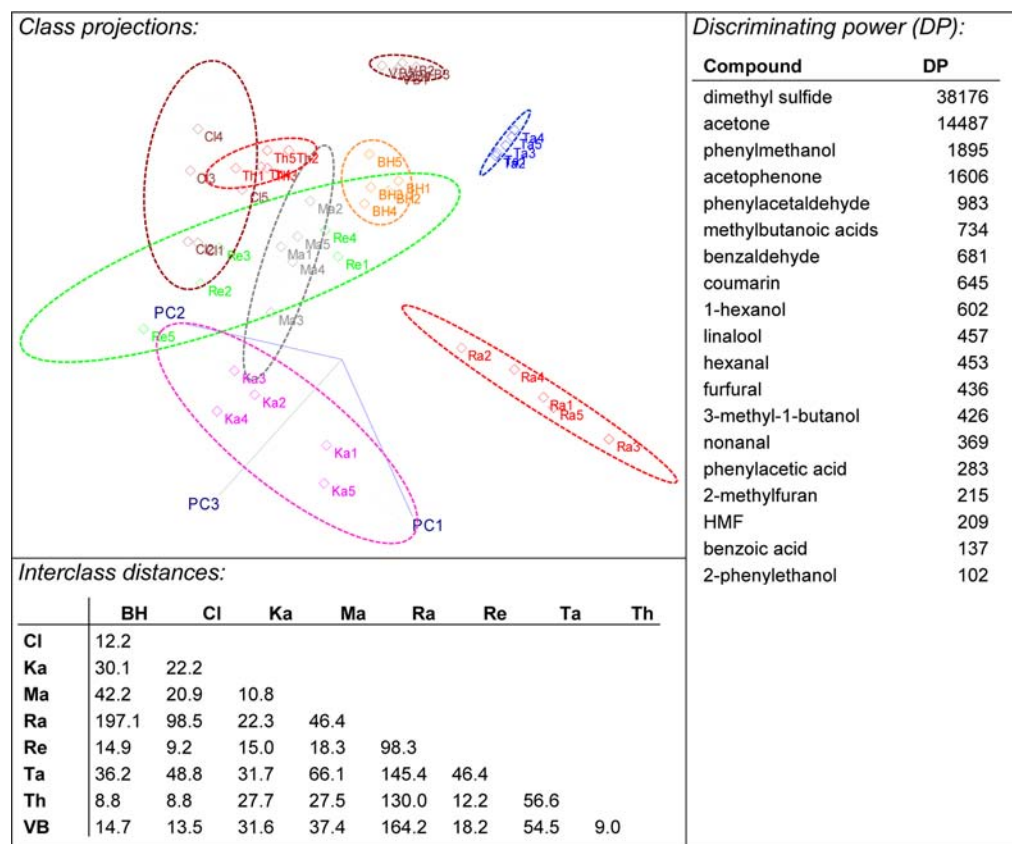


Figure 2. SIMCA multivariate analysis of SIFT-MS SIM data from New Zealand monofloral honeys (see Table 2). Methanol, ethanol, and acetaldehyde have been removed. The key to the honey types shown in the interclass distances is given in Table 1. See the text for further details.

Selected Ion Mode (SIM) Data. Table 3 lists 22 compounds that were targeted in this study and the reagent ion–product ion combinations that were used to detect them. Because of a lack of information on the volatile components of New Zealand monofloral honeys, the compounds in the method were selected based on what could be identified in SIFT-MS mass scans and the GC-MS thermal desorption data reported in Table 2 for these honeys, together with other volatile compounds that have been previously identified in international studies on various honey types (see Table 2). The product ions identifying the volatiles that perform best in the SIFT-MS headspace analyses are highlighted in bold. These product ion masses are assumed to have the least interference, as determined by them yielding the lowest concentration value (calculated for each product ion). Remaining possible overlaps with these well-performing masses are also indicated in the potential interferents column. Note that unresolved conflicts mean the reported concentration provides an upper limit for that compound. The concentrations determined for each honey are given in Table 4, together with the measurement uncertainty (one standard deviation of the mean). Although the number of samples in the present study was small, it is instructive to demonstrate how the different distributions of volatiles in the aromas of each sample of the pooled honey batches differ by using multivariate statistical analysis provided by the SIMCA algorithm in Infometrix Pirouette software.^{40,41} The application of this software to SIFT-MS has been fully described elsewhere.⁴² The results of this analysis is shown in Figure 1 for all volatiles and in Figure 2 where three of the most volatile compounds, methanol, ethanol, and acetaldehyde

have been removed. All classes are well resolved because the interclass distances are greater than three.⁴¹ All of the honey samples are still readily distinguished from each other.

A principal object of this study was to examine the ability of SIFT-MS to distinguish pure manuka honey from common impurities: beech honeydew and rewarewa honey in the South and North Islands of New Zealand, respectively. The preliminary results presented here suggest that the SIFT-MS technique, combined with multivariate statistical analysis, provides a possible simple means of assessing each honey type evaluated. An obvious next step is to increase the sample size by examining honey from even larger numbers of apiaries and different growing seasons.

Discrimination can be performed reliably using either a “black box” approach with any one of the SIFT-MS reagent ions or via quantification of specified marker compounds known to be present in honey volatiles.

Although good discrimination between the nine New Zealand honeys was found using only those volatiles that were prominent in other honey studies (Table 2), the most effective discriminators in the current study using all the volatiles (Figure 1) were those compounds with the highest volatility: methanol, ethanol and acetaldehyde. These compounds may, in the long term, prove to be less reliable markers for honeys as the fermentation status and postharvesting treatment of the honey by the beekeeper may contribute a significant component of the variability and this needs to be tested. When these three highest volatility compounds were removed from the statistical analysis, dimethyl sulfide was the volatile having the highest discriminating power (Figure 2). Dimethyl sulfide has been identified in

14 honeys in Lithuania³⁴ and has also been shown to be a major contributor in distinguishing between 22 different honey samples from 8 monofloral honeys.⁴³ It is also an important discriminator in distinguishing between orange and lemon honeys.⁴⁴ It is noteworthy that dimethyl sulfide has the highest volatility of the compounds included in Figure 2.

SIFT-MS is a simple method to implement, and in this exploratory study, it was used to distinguish between different New Zealand honeys based on their aroma. We also note that although the batches of honey sampled here represent a significant number of top quality honeys from reputable beekeepers, this does not provide a guarantee that the next batches would have identical profiles. Further studies are needed. Future studies may improve the procedure by concentrating on stable markers of specific floral nectars once these have been identified. These markers could then act as discriminators of origin and should also be independent of the honeys' processing history.

■ ASSOCIATED CONTENT

● Supporting Information

Example of SIFT-MS full mass scan for New Zealand white clover and manuka honeys using the reagent ion, NO⁺. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Department of Chemistry, University of Canterbury, PB 4800, Christchurch, New Zealand. Phone: 64-3 338 6701. Fax: 64-3-338-6704. E-mail: murray.mcewan@canterbury.ac.nz.

Notes

The authors declare no competing financial interest.

■ REFERENCES

- (1) Molan, P. C.; Russell, K. M. Non-peroxide antibacterial activity in some New Zealand honeys. *J. Apic. Res.* **1988**, *27*, 62–67.
- (2) Russell, K. M.; Molan, P. C.; Wilkins, A. L.; Holland, P. T. The identification of some antibacterial constituents of New Zealand manuka honey. *J. Agric. Food Chem.* **1988**, *38*, 10–13.
- (3) Somal, N. A.; Coley, K. E.; Molan, P. C.; Hancock, B. M. Susceptibility of *helicobacter pylori* to the antibacterial activity of manuka honey. *J. R. Soc. Med.* **1994**, *87*, 9–12.
- (4) Weston, R. J.; Brocklebank, L. K.; Lu, Y. Identification and quantitative levels of antibacterial components of some New Zealand honeys. *Food Chem.* **2000**, *70*, 427–435.
- (5) Cooper, R. A.; Molan, P. C.; Krishnamoorthy; Harding, K. K. Manuka honey used to heal a recalcitrant surgical wound. *Eur. J. Clin. Microbiol. Infect. Dis.* **2001**, *20*, 758–759.
- (6) Tonks, A. J.; Dudley, E.; Porter, N. G.; Parton, J.; Brazier, J.; Smith, E. L.; Tonks, A. A 5.8-kDa component of manuka honey stimulates immune cells via TLR4. *J. Leukocyte Biol.* **2007**, *82*, 1147–1155.
- (7) Mavric, E.; Wittmann, S.; Barth, G.; Henle, T. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. *Mol. Nutr. Food Res.* **2008**, *52*, 483–489.
- (8) Inoue, K.; Murayama, S.; Seshimo, F.; Takebas, K.; Yoshimura, Y.; Nakazawa, H. Identification of phenolic compound in manuka honey as specific superoxide anion radical scavenger using electron spin resonance (ESR) and liquid chromatography with coulometric array detection. *J. Sci. Food Agric.* **2005**, *85*, 872–878.
- (9) Jenkins, R.; Burton, N.; Cooper, R. Manuka honey inhibits cell division in methicillin-resistant *Staphylococcus aureus*. *J. Antimicrob. Chemother.* **2011**, *66*, 2536–2542.

(10) Jenkins, R.; Burton, N.; Cooper, R. Effect of manuka honey on the expression of universal stress protein A in methicillin-resistant *Staphylococcus aureus*. *Int. J. Antimicrob. Agents* **2011**, *37*, 373–376.

(11) Codex Alimentarius for Honey at <http://www.beekeeping.com> (accessed January 17, 2012).

(12) Ruoff, K.; Luginbuhl, W.; Kunzli, R.; Iglesias, M. T.; Bogdanov, S.; Bosset, J. O.; von der Ohe, K.; von der Ohe, W.; Amado, R. Authentication of the botanical and geographical origin of honey by mid-infrared spectroscopy. *J. Agric. Food Chem.* **2006**, *54*, 6873–6880.

(13) Kaskoniene, V.; Venskutonis, P. R. Floral markers in honey of various botanical and geographical origins: a review. *Comp. Rev. Food Sci. Food Saf.* **2010**, *9*, 620–634.

(14) D'Arcy, B. R.; Rintoul, G. B.; Rowland, C. Y.; Blackman, A. J. Composition of Australian honey extractives. I. norisoprenoids, monoterpene and other natural volatiles from blue gum (*Eucalyptus leucocylon*) and yellow box (*Eucalyptus melliodora*) honeys. *J. Agric. Food Chem.* **1997**, *45*, 1834–1843.

(15) Perez, R. A.; Sanchez-Brunete, C.; Calvo, R. M.; Tadeo, J. L. Analysis of volatiles from Spanish honeys by solid phase micro-extraction and gas chromatography-mass spectrometry. *J. Agric. Food Chem.* **2002**, *50*, 2633–2637.

(16) Bonvehi, J. S.; Ventura Coll, F. Flavour index and aroma profiles of fresh and processed honeys. *J. Sci. Food Agric.* **2003**, *83*, 275–282.

(17) Jerkovic, I.; Marijanovic, Z.; Malenica Staver, M. Screening of natural organic volatiles from *Prunus mahaleb* L. honey: coumarin and vomifolol as nonspecific biomarkers. *Molecules* **2011**, *16*, 2507–2518 and references contained therein.

(18) Bianchi, F.; Mangia, A.; Mattarozzi, M.; Musci, M. Characterization of the volatile profile of thistle honey using headspace solid-phase microextraction and gas chromatography-mass spectrometry. *Food Chem.* **2011**, *129*, 1030–1036.

(19) Manyi-Loh, C. E.; Ndip, R. N.; Clarke, A. M. Volatile compounds in honey: a review on their involvement in aroma, botanical origin determination and potential biomedical activities. *Int. J. Mol. Sci.* **2011**, *12*, 9514–32.

(20) Anklam, E. A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chem.* **1998**, *63*, 549–562.

(21) Visser, F. R.; Allen, J. M.; Shaw, G. J. The effect of heat on the volatile flavour fraction from a unifloral honey. *J. Api. Res.* **1988**, *27*, 175–181.

(22) Wilkins, A. L.; Lu, Y.; Molan, P. C. Extractable organic substances from 1989–90 season unifloral New Zealand manuka (*Leptospermum scoparium*) honeys. *J. Apic. Res.* **1993**, *32*, 3–9.

(23) Tan, S. T.; Holland, P. T.; Wilkins, A. L.; Molan, P. C. Extractives from New Zealand honeys. I. White clover, manuka and kanuka unifloral honeys. *J. Agric. Food Chem.* **1988**, *36*, 453–460.

(24) Adams, C. J.; Manley-Harris, M.; Molan, P. C. The origin of methylglyoxal in New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydr. Res.* **2009**, *344*, 1050–1053.

(25) Fearnely, L.; Greenwood, D. R.; Schmitz, M.; Schlothauer, R. C.; Loomes, K. M. Compositional analysis of manuka honeys by high-resolution mass spectrometry: identification of a manuka-enriched archetypal molecule. *Food Chem.* **2012**, *132*, 948–953.

(26) Daher, S.; Gulacar, F. O. Identification of new aromatic compounds in the New Zealand manuka honey by gas chromatography-mass spectrometry. *E-J. Chem.* **2010**, *7*, S7–S14.

(27) Freeman, C. G.; McEwan, M. J. Rapid analysis of trace gases in complex mixtures using SIFT-MS. *Aust. J. Chem.* **2002**, *55*, 491–494.

(28) Smith, D.; Spanel, P. Selected ion flow tube mass spectrometry for on-line trace gas analysis. *Mass Spectrom. Rev.* **2005**, *24*, 661–700.

(29) Syft preliminary data obtained from TD-GCMS measurements.

(30) Shimoda, M.; Wu, Y.; Osajima, Y. Aroma compounds from aqueous solution of haze (*Rhus succedanea*) honey determined by adsorptive column chromatography. *J. Agric. Food Chem.* **1996**, *44*, 3913–3918.

(31) Moreira, R. F. A.; Trugo, L. C.; Pietrolungo, M.; De Maria, C. A. B. Flavor composition of cashew (*Anacardium occidentale*) and

marmeleiro (*Croton species*) honeys. *J. Agric. Food Chem.* **2002**, *50*, 7616–7621.

(32) Radovic, B. S.; Careri, M.; Mangia, A.; Musci, M.; Gerboles, M.; Anklam, E. Contribution of dynamic headspace GC-MS analysis of aroma compounds to authenticity testing of honey. *Food Chem.* **2001**, *72*, 511–520.

(33) Alissandrakis, E.; Kibaris, A. C.; Tarantilis, P. A.; Harizanis, P. C.; Polissiou, M. Flavor compounds of Greek cotton honey. *J. Sci. Food Agric* **2005**, *85*, 1444–1452.

(34) Kaskoniene, V.; Ventskutonis, R.; Ceksteryte, V. Composition of volatile compounds of honey of various floral origin and beebread collected in Lithuania. *Food Chem.* **2008**, *111*, 988–997.

(35) Piasenzotto, L.; Gracco, L.; Conte, L. Solid phase micro-extraction (SPME) applied to honey quality control. *J. Sci. Food Agric* **2003**, *83*, 1037–1044.

(36) Odeh, I.; Abu-Lafi, S.; Dewik, H.; Al-Najjar, I.; Imam, A.; Dembitsky, V. M.; Hanus, L. O. A variety of volatile compounds as markers in Palestinian honey from *Thymus capitatus*, *Thymelaea hirsute*, and *Tolpis virgate*. *Food Chem.* **2007**, *101*, 1393–1397.

(37) Alissandrakis, E.; Tarantilis, P. A.; Harizanis, P. C.; Polissiou, M. Comparison of the volatile composition in thyme honeys from several origins in Greece. *J. Agric. Food Chem.* **2007**, *55*, 8152–8157.

(38) Cajka, T.; Hajslova, J.; Pudil, F.; Riddellova, K. Traceability of honey origin based on volatiles pattern processed by artificial neural networks. *J. Chromatogr., A.* **2009**, *1216*, 1458–1462.

(39) Bouseta, S.; Collin, S. Optimized Likens-Nickerson methodology for quantifying honey flavors. *J. Agric. Food Chem.* **1995**, *43*, 1890–1897.

(40) Wold, S. 1976. Pattern recognition by means of disjoint principal components modules. *Pattern Recognit.* **1976**, *8*, 127–129.

(41) Kvalheim, O. M.; Karstang, T. V. SIMCA-Classification by Means of Disjoint Cross Validated Principal Component Models, in *Multivariate Pattern Recognition in Chemometrics, Illustrated by Case Studies*; Brereton, R. G., Ed.; Elsevier: Amsterdam, 1992.

(42) Langford, V. S.; Reed, C. J.; Milligan, D. B.; McEwan, M. J.; Barringer, S. A.; Harper, W. J. Headspace analysis of Italian and New Zealand Cheeses. *J. Food Sci.* **2012**, DOI: 10.1111/j.1750-3841.2012.02730x.

(43) Soria, A. C.; Martinez-Castro, I.; Sanz, J. Some aspects of dynamic headspace analysis of volatile components in honey. *Food Res. Int.* **2008**, *41*, 838–848.

(44) Kadar, M.; Juan-Borras, M.; Carot, J. M.; Domenech, E. Volatile fraction composition and physicochemical parameters as tools for the differentiation of lemon blossom honey and orange blossom honey. *J. Sci. Food Agric.* **2011**, *91*, 2768–2776.