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Non-targeted metabolomic strategy for characterization of botanical origin of honey samples using headspace gas chromatography – ion mobility spectrometry

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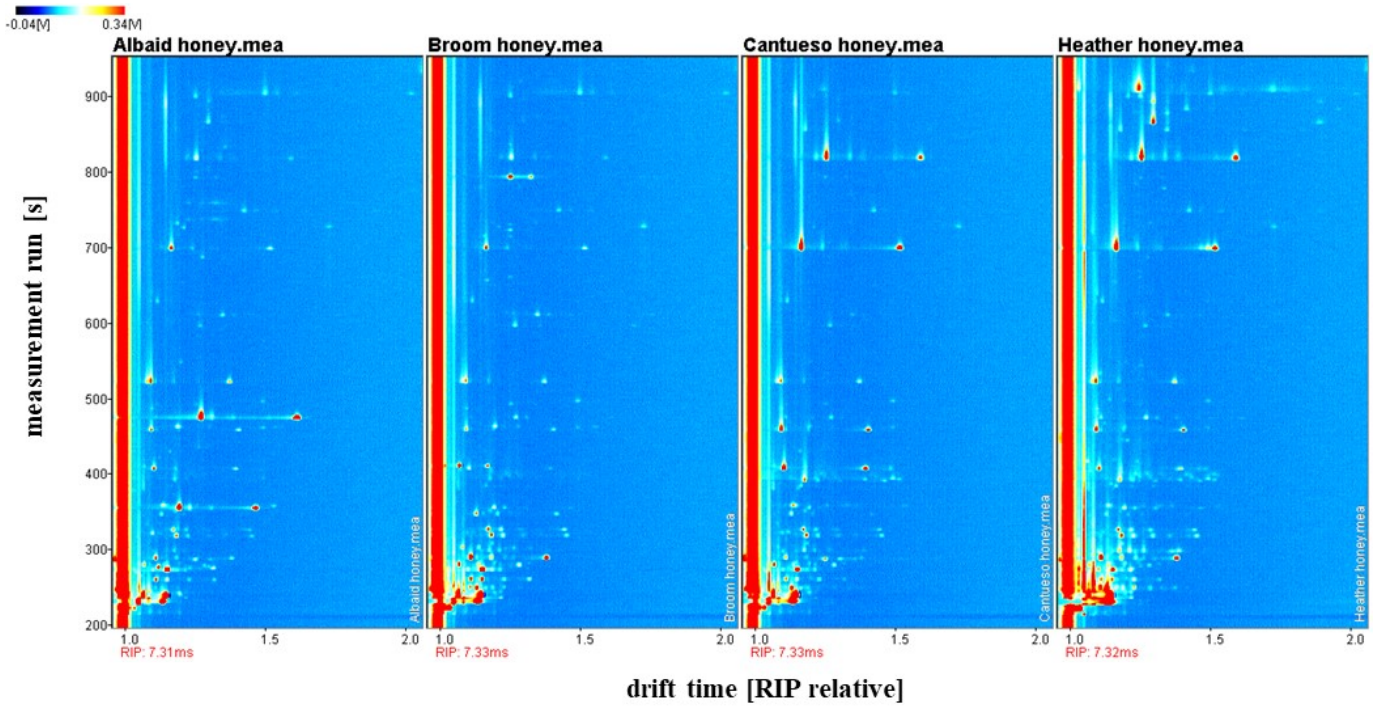
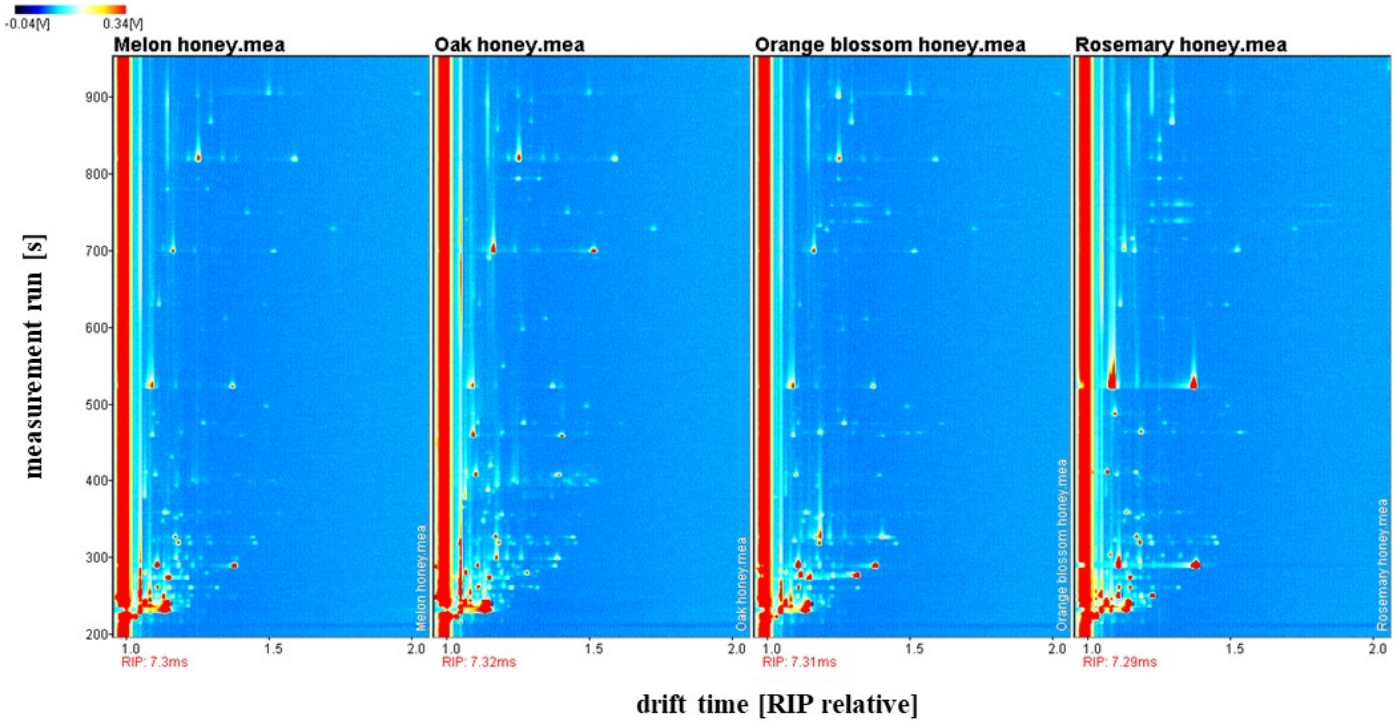
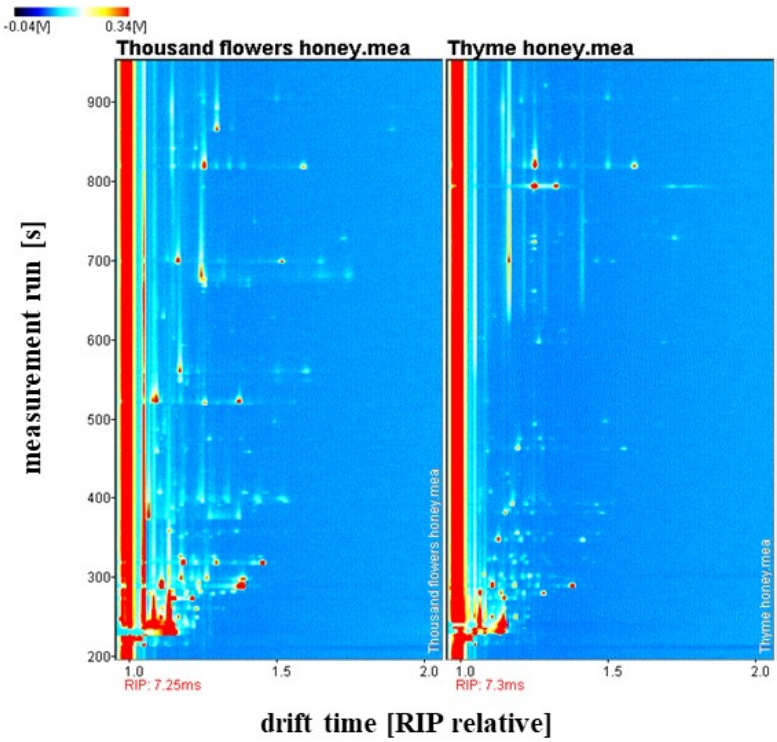


Supplemental Figure S1. Spectra obtained for albaid, broom, cantueso and heather honeys.



Supplemental Figure S2. Spectra obtained for melon, oak, orange blossom and rosemary honeys.



Supplemental Figure S3. Spectra obtained for thousand flowers and thyme honeys.

Supplemental Table S1. Selection of the pre-treatment of the HS-GC-IMS data and chemometrics model for honey classification according to its floral origin. The training set was composed of 71 honey samples used to build five[a] class calibration models, and the validation set consisted of 18 samples.

Model information					CR (%)		Sensitivity (%)	Precision (%)
Scaling[b]	Components	Validation	R2Y(cum)	Q2(cum)	Calibration	Validation	Validation	Validation
Raw data								
UV	4 + 9 + 0	0.743	0.993	0.796	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)
UVN	4 + 9 + 0	0.921	0.919	0.810	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)
Par	4 + 9 + 0	0.828	0.906	0.767	100	94.44	A (100), OB (66.67), TF (100), O (100), R (100)	A (66.67), OB (66.67), TF (83.33), O (85.71), R (66.67)
ParN	4 + 9 + 0	0.950	0.902	0.768	98.59	94.44	A (100), OB (66.67), TF (100), O (100), R (100)	A (66.67), OB (66.67), TF (83.33), O (85.71), R (66.67)
Ctr	4 + 6 + 0	0.865	0.739	0.570	94.37	88.89	A (100), OB (100), TF (80), O (100), R (100)	A (50), OB (60), TF (66.67), O (75), R (33.33)
Freeze	4 + 9 + 0	0.743	0.933	0.796	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)
UV + log transformation	4 + 8 + 0	0.736	0.929	0.774	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)
UVN + log transformation	3 + 9 + 0	0.988	0.730	0.573	94.37	94.44	A (100), OB (66.67), TF (100), O (100), R (100)	A (66.67), OB (66.67), TF (83.33), O (85.71), R (66.67)
Par + log transformation	4 + 7 + 0	0.745	0.911	0.773	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)
ParN + log transformation	4 + 8 + 0	0.736	0.929	0.774	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)
Ctr + log transformation	4 + 9 + 0	0.822	0.926	0.798	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)
Freeze + log transformation	4 + 8 + 0	0.736	0.929	0.774	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)
Normalized data respect to the reactant ion peak intensity								
UV	4 + 10 + 0	0.773	0.943	0.801	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)
UVN	4 + 12 + 0	0.942	0.949	0.845	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)
Par	4 + 9 + 0	0.831	0.908	0.754	100	94.44	A (100), OB (66.67), TF (100), O (100), R (100)	A (66.67), OB (66.67), TF (83.33), O (85.71), R (66.67)
ParN	4 + 10 + 0	0.956	0.915	0.768	100	94.44	A (100), OB (66.67), TF (100), O (100), R (100)	A (66.67), OB (66.67), TF (83.33), O (85.71), R (66.67)
Ctr	4 + 2 + 0	0.759	0.586	0.457	91.55	88.99	A (100), OB (100), TF (80), O (100), R (100)	A (50), OB (60), TF (66.67), O (75), R (33.33)
Freeze	4 + 10 + 0	0.773	0.943	0.801	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)
UV + log transformation	**5 + 10 + 0**	**0.967**	**0.951**	**0.851**	**100**	**100**	**A (100), OB (100), TF (100), O (100), R (100)**	**A (100), OB (100), TF (100), O (100), R (100)**
UVN + log transformation	4 + 2 + 0	0.990	0.605	0.461	88.83	83.33	A (100), OB (100), TF (80), O (83.33), R (50)	A (40), OB (50), TF (57.14), O (62.50), R (25)
Par + log transformation	4 + 11 + 0	0.827	0.961	0.837	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)
ParN + log transformation	4 + 2 + 0	0.992	0.622	0.470	90.14	83.33	A (100), OB (100), TF (80), O (83.33), R (50)	A (40), OB (50), TF (57.14), O (62.50), R (25)
Ctr + log transformation	4 + 11 + 0	0.851	0.961	0.843	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)
Freeze + log transformation	4 + 9 + 0	0.767	0.911	0.791	100	100	A (100), OB (100), TF (100), O (100), R (100)	A (100), OB (100), TF (100), O (100), R (100)

[a]Classes are albaid (A), orange blossom (OB), thousand flowers (TF), other (O) and rosemary (R) botanical origin honeys.

[b]Ctr, centering; Log, logarithmic transformation; Par, Pareto; ParN, Pareto none; UV, unit variance; UVN, unit variance none.

Bold letter indicates the pre-treatments and chemometric models finally chosen for further studies.

Supplemental Table S2. VOCs identified in different honey samples in other countries.

VOC[a] identified	Honey type investigated	Country	Reference
2-butanone, ethyl acetate, 2-pentanone, valeraldehyde, 3-methyl-1-butanol, 2-hexanone, hexanal, trans-2-hexenal, 2-heptanone, heptanal, **benzaldehyde**, 6-methyl-5-hepten-2-one, octanal, **linalool**, **nonanal**.	Thousand flowers, rosemary, albaid, orange blossom, honey broom, thyme, heather, melon, Spanish lavender and oak	Spain	This work
octanal, benzene acetaldehyde,1-octanol, 2-methoxyphenol, **nonanal**, 2-H-1-benzopyran-2-one	Alfalfa, sunflower, white clover, carob, calden	Argentina	37
Furfural, **benzaldehyde**, hotrienol, benzeneacetaldeyde, octanoic acid, nonanoic acid, n-decanoic acid, benzoic acid, **nonanal**, acetic acid, decanal, **linalool**, phenylethyl alcohol, dodecanoic acid, tetradecanoic acid, terpineol	Acacia, apple dandelion, chesnut, honeydew, multifloral, rododendron	Italy	38
Methyl octanoate, methyl nonanoate, methyl decanoate, methyl hexadecanoate, furan-2-carbaldehyde, **benzaldehyde**, 2-phenylacetaldehyde, **nonanal**, decanal, 4-isopropylbenzaldehyde, 2-phenylethan-1-ol, carvacrol, 1-phenylbutane-2,3-dione, thymoquinone, 3-hydroxy-4-phenyl-2-butanone, butane, heptane, octane, nonane, undecane, isobutyronitrile, 2-methylbutanenitrile, 3-methylbutanenitrile, 2-phenylacetonitrile, methylbenzene, p-cymene, 2,5-diethyltetrahydrofuran, methyl 2-oxo-2-phenylacetate, 1,1,5-trimethyl-1,2-dihydronaphthalene, 1-methyl-4-(prop-1-en-2-yl)benzene.	Thyme	Greece	39
3-caren-2-ol, p-cymene, linalool oxide, lilac alcohol, lilac aldehyde, nerolidol oxide	Eucalyptus, citrus, lavender	Spain	40
Benzaldehyde, α-pinene, benzene acetaldehyde, acetophenone, cis- and furanoid, UI 8, 1,2-dihydro-1,1,6-trimethyl-naphtalene, α-eudesmol, β-eudesmol, aromadendrene, benzyl salicylate.	Bell heather, chestnut, eucalyptus, incense, sunflower	Portugal	41

[a] VOC: Volatile Organic Compound

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