L’origine botanica dei mieli italiani: HS-GC-IMS e HS-SPMEGC-IMS-MS per la decifrazione dei marcatori chiave

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Workshop Lab Service Analytica, 22 Aprile 2026

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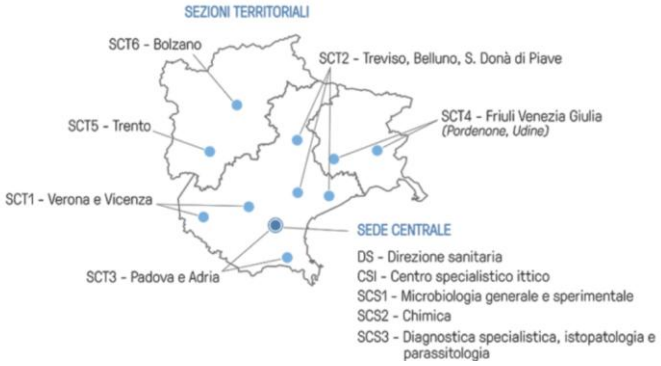
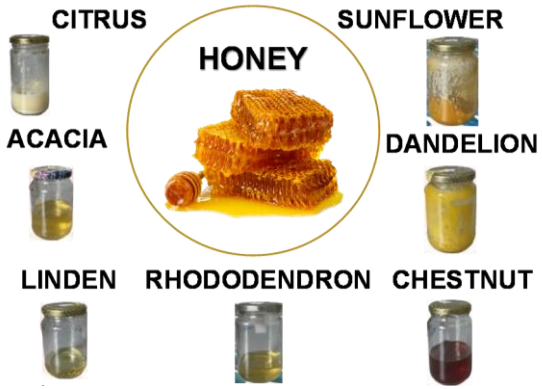
- **Official controls on Food and livestock animals**
- **Analytical and Microbiological services for Industry and Farm support**
- **Research**



Monofloral honey and floral origin

Monofloral honey is a type of honey that has a distinctive flavor because it is mainly generated from the nectar of a single plant species.

In other words, bees producing monofloral honey mainly collect nectar and pollen from a specific flower.



- the use <u>of additives and colorants to misrepresent the true botanical origin of honey</u>, mask the true geographical origin of honey by falsifying traceability information, and remove pollen.

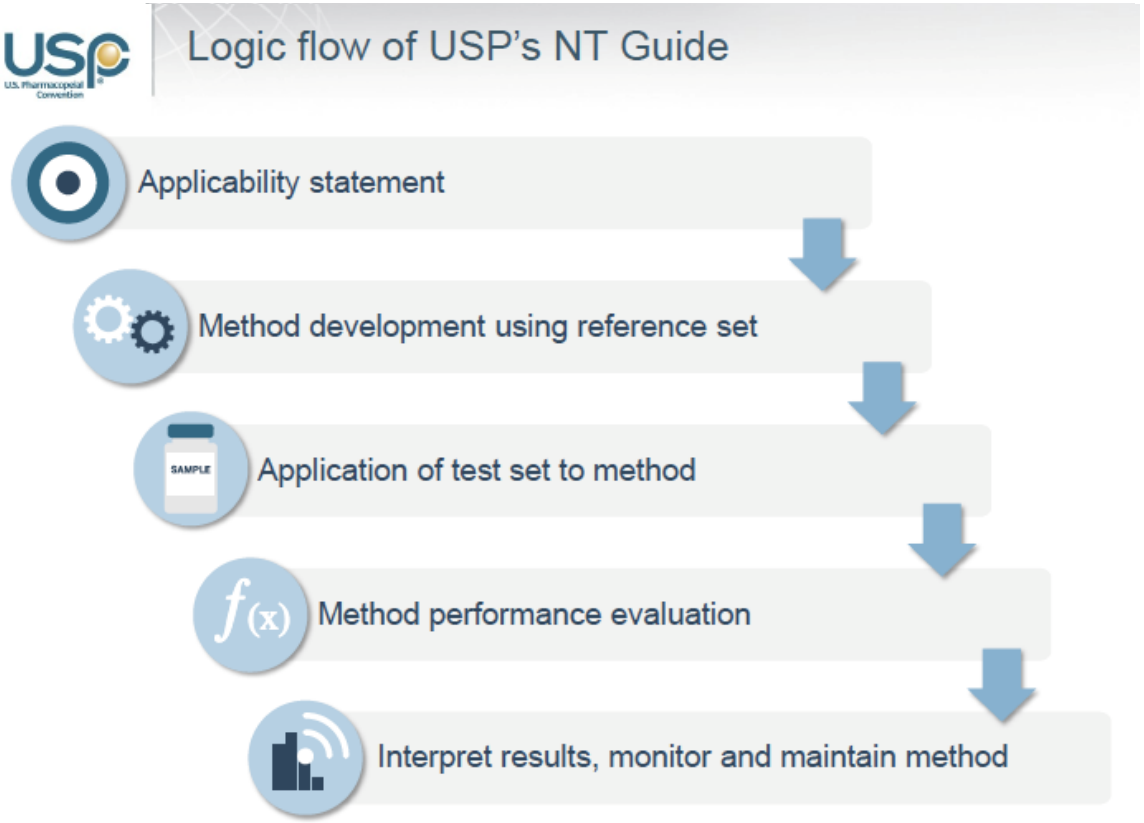
Breakfast directive: Directive (UE) 2024/1438

- preserves European beekeeping and the quality of European honey as an unprocessed raw agricultural product; it imposes stricter labeling and composition requirements for breakfast products to combat “food fraud” in the breakfast sector
- amends Council Directive 2001/110/EC on honey and other directives concerning jams, marmalades, jellies, and chestnut creams

Breakfast directive: Direttiva (UE) 2024/1438

- The country of origin of the honey must be clearly visible on the label
- The countries of origin will be listed on the label in descending order by weight;
- The label will also include the percentage each country represents in the blend, thereby increasing transparency for consumers
- Under the new regulation, honey labels must now accurately state the product's origin, floral source, and processing methods.
- The directive also requires that any indication of the geographical origin or botanical source of the honey must be verifiable
- An EU traceability system for honey is required

Development and validation of non-targeted methods in food authentication: Guidelines to standardize non-targeted methods



Development and validation of non-targeted methods in food authetication

Experimental design

Heterogeneous reference set of samples

Testing analytical tecniques

Acquisition of the fingerprints including pooled QC

Pre-processment and verification of data quality

Machine Learning

AUTOMATED SYSTEM

Authetication

Validation of the method

Late stage validation

Annotation of *important features*

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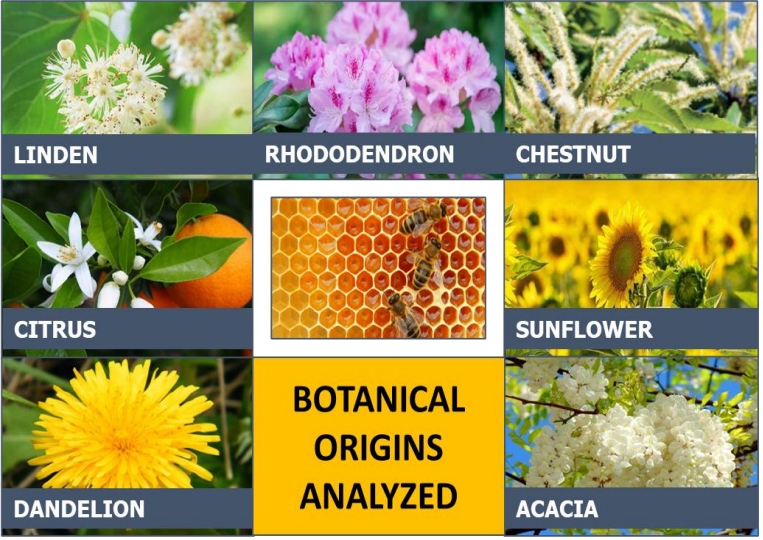
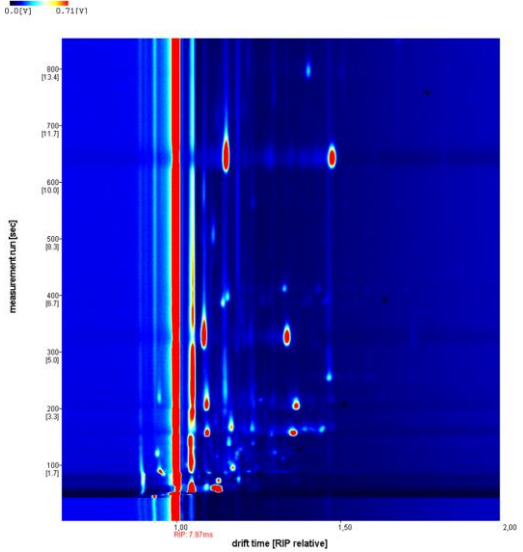
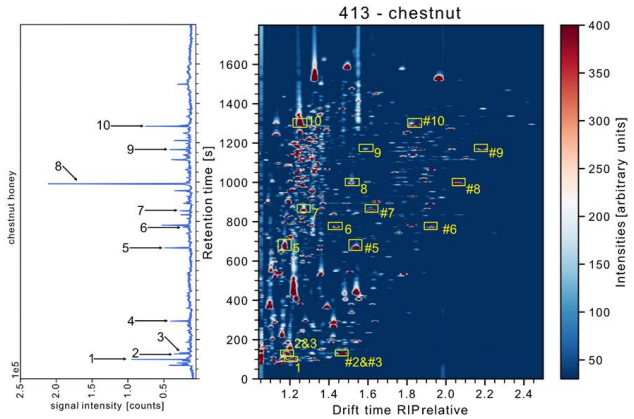
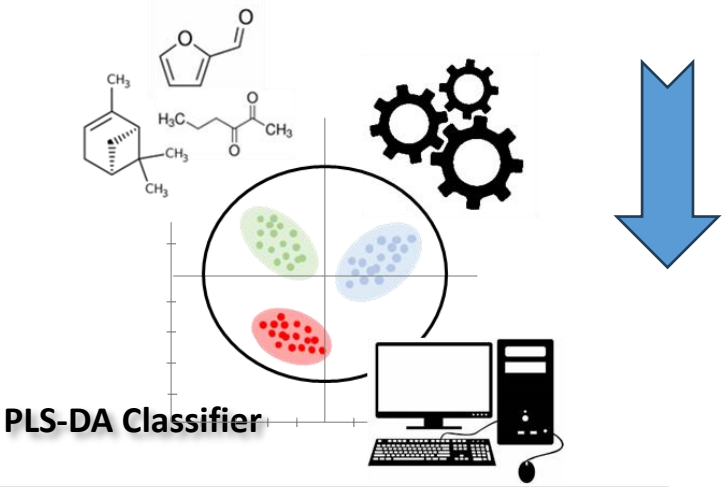




- **Botanical origins:** Acacia, Chestnut, Citrus, Dandelion, Linden, Rhododendron and Sunflower;
- **Harvest seasons:** 2022- 2023.

- **Analysis of 136 samples by GC-IMS;**
- **Within a time-span of a year;**
- **2 operators**



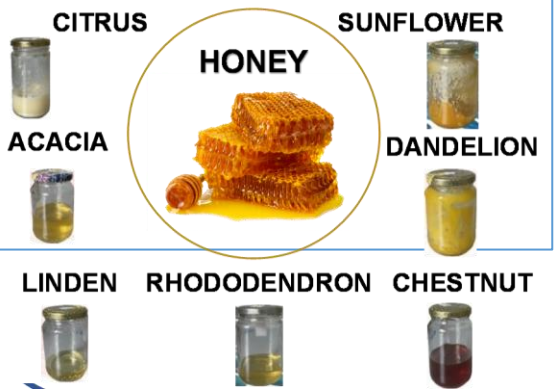


- **Annotation of the most distinct volatiles of each botanical floral source by SPME-HS-GC-IMS-MS**

- **Cross-validation on training set (n=107)**
- **Validation on withheld test set (n=29)**

The reference samples:

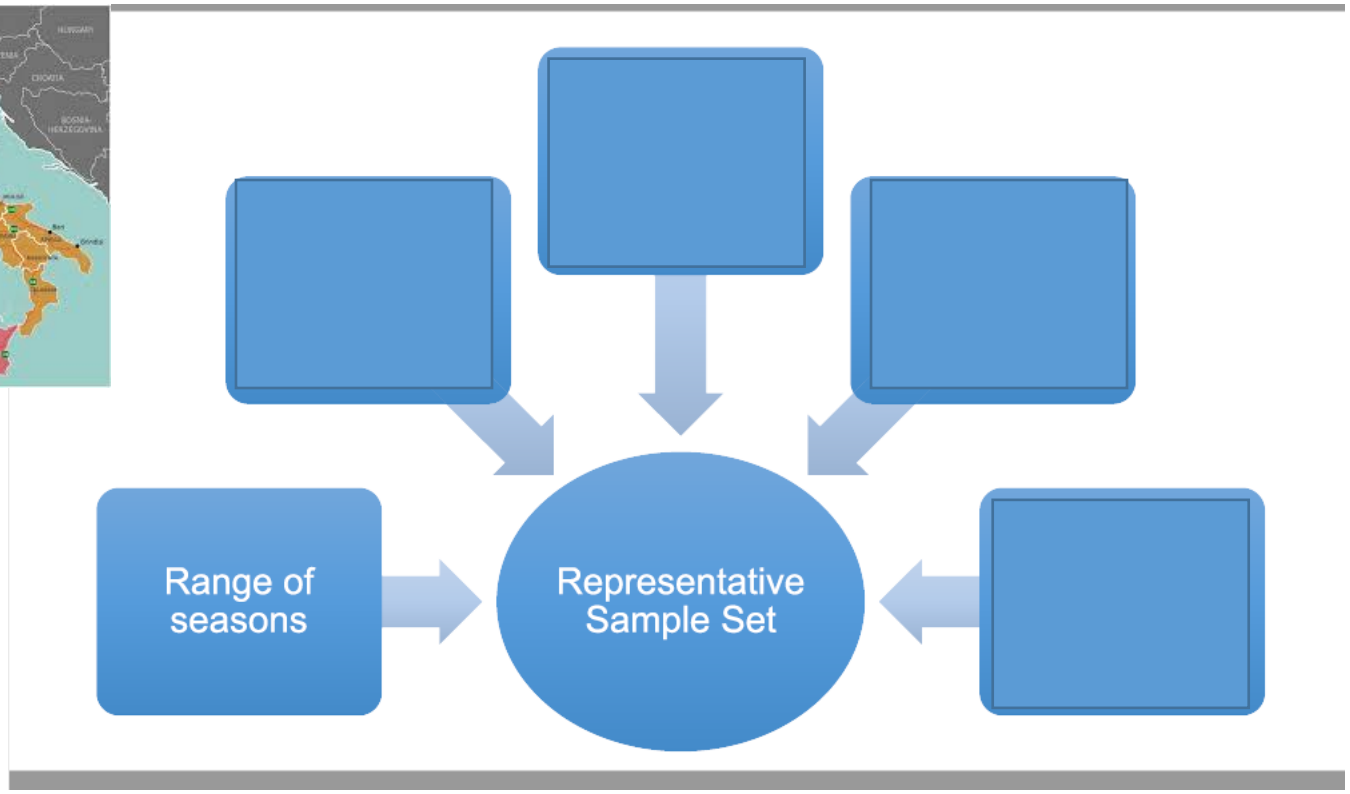
- Total number of samples: 136
- Two independent batches
- Two harvest seasons
- 7 botanical origins



- Authenticated by physico-chemical and sensory analyses

OSSERVATORIO NAZIONALE MIELE

The reference set samples of the non-targeted method development in food autheticity



Terry F. McGrath et al. Trends in Food Science & Technology, Volume 76, 2018, 38-55

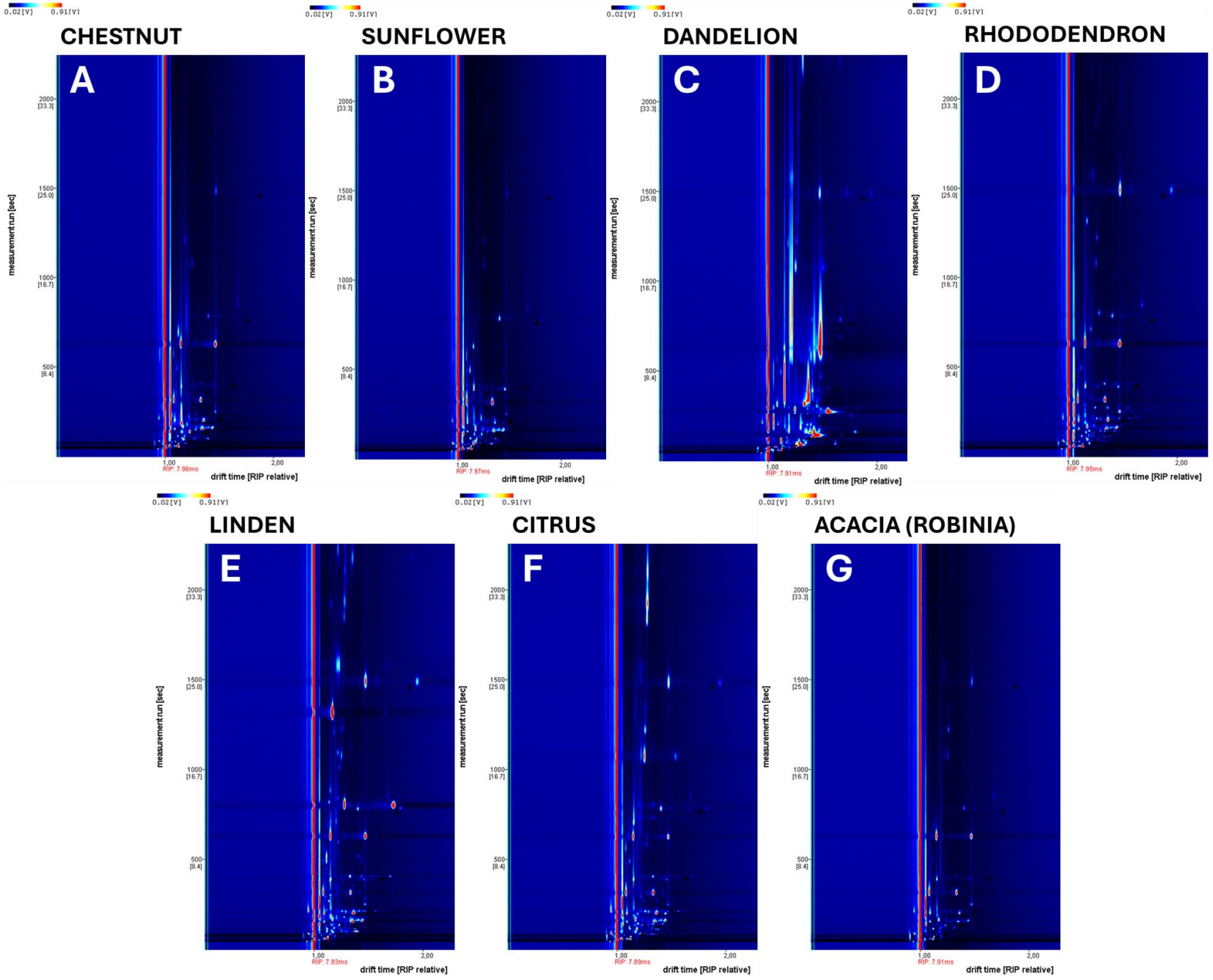
1.500 g ± 0.001 g of each honey sample were weighed into a 20 mL vial, sealed with a cap, and placed in the autosampler tray.

Incubation was performed at 60 °C for 15 min.

A flow ramp was set as follows: start flow at 5 mL/min; increase to 25 mL/min for the next 40 min; finally, decrease the flow for the next 5 min to the default pressure (5 mL/min); end the program at 45 min. Spectral averaging was set to six scans. FS-SE-54-CB-0.5 column

The drift tube was kept at a steady temperature of 45 °C with a nitrogen gas flux of 150 mL/min flowing in the opposite direction from the sample (drift gas).

Total analysis time on the autosampler was set to 47 min between injections to allow pressure equilibrium to be reached in the FlavourSpec injector at the initial flow rate of 5 mL/min. Total run time was 2400 s.


CHESTNUT
A
SUNFLOWER
B
DANDELION
C
RHODODENDRON
D
LINDEN
E
CITRUS
F
ACACIA (ROBINIA)
G
0.02[V]
0.91[V]
measurement run [sec]
drift time [RIP relative]
RIP: 7.96ms
RIP: 7.97ms
RIP: 7.91ms
RIP: 7.95ms
RIP: 7.93ms
RIP: 7.89ms
RIP: 7.91ms

- CITRUS HONEY
- SUNFLOWER HONEY
- CHESTNUT HONEY
- DANDELION HONEY
- RHODODENDRON HONEY
- LINDEN HONEY
- ACACIA HONEY

DATA SET

PREPROCESSED DATA SET

EXPLORATORY DATA ANALYSIS

PLS-DA

PCA

TRAINING SET

TEST SET

Use as training set

Learning algorithms:
- ✓ **Random forest**
- ✓ **LASSO**
- ✓ **PLS-DA**

Hyper-parameter optimization and features selection

TRAINED MODELS

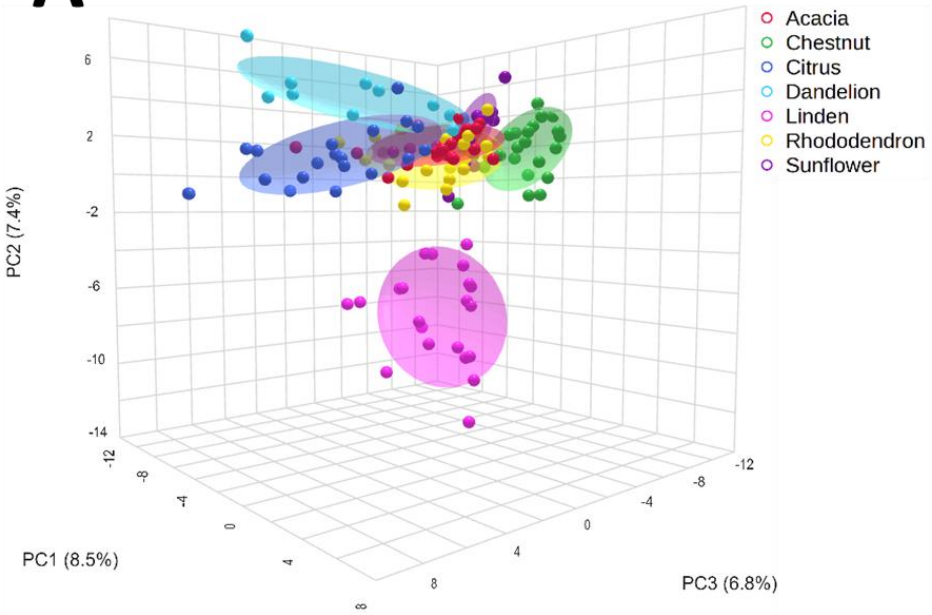
PLS-DA

EVALUATION OF THE MODEL PERFORMANCE

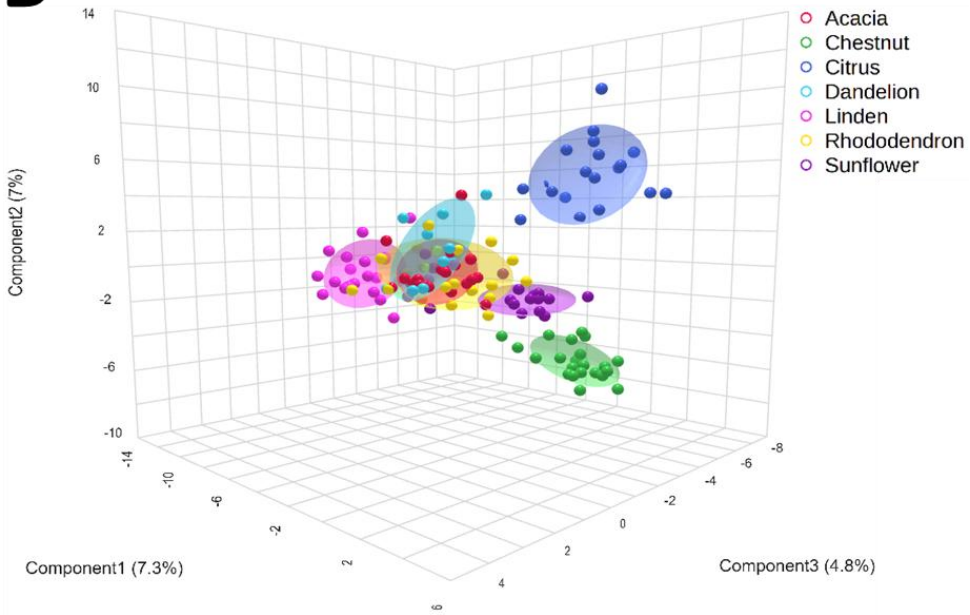
1. Cross-validation
2. Permutation test
3. Resubstitution

- ✓ **AUC**
- ✓ **ACCURACY**
- ✓ **KAPPA**
- ✓ **SENSITIVITY**
- ✓ **SPECIFICITY**

A


Acacia
Chestnut
Citrus
Dandelion
Linden
Rhododendron
Sunflower
PC2 (7.4%)
PC1 (8.5%)
PC3 (6.8%)

B

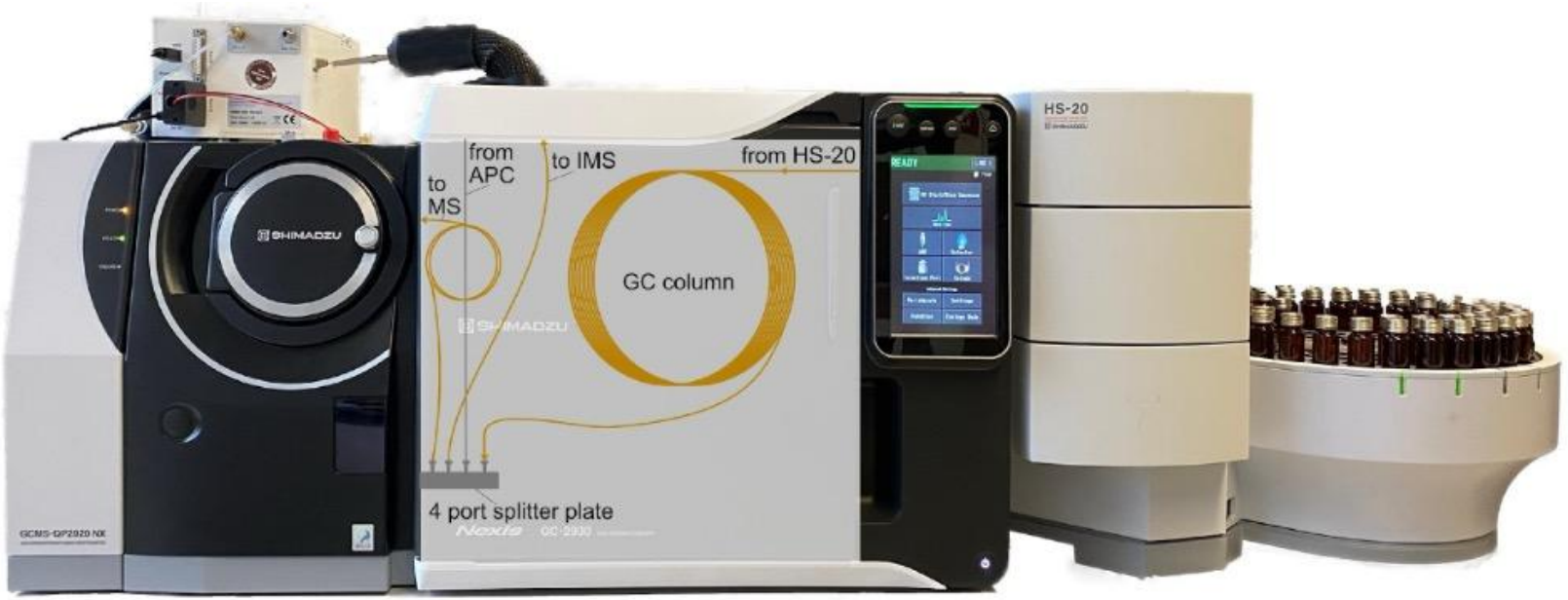

Acacia
Chestnut
Citrus
Dandelion
Linden
Rhododendron
Sunflower
Component2 (7%)
Component1 (7.3%)
Component3 (4.8%)

MODEL PERFORMANCE OF THE BEST MODEL

Classifier	Classifier evaluation	AUC	Accuracy	Kappa	Sensitivity	Specificity
Best model PLS-DA	Cross validation	96.73%	99.07%	98.90%	98.21%	99.83%
	Withheld validation set	83.33%	93.10%	91.80%	90.00%	98.75%

Sample	Botannical origin proposed by classifier	Actual botannical origin
230926_145536_Honey_4_og	Acacia	Acacia
230928_051305_Honey_80_og	Acacia	Acacia
230929_120038_Honey_89_og	Acacia	Acacia
240125_213229_Honey_133_og_23	Acacia	Acacia
240126_170252_Honey_220_og_23	Acacia	Acacia
231003_003142_Honey_174_og	Chestnut	Chestnut
240127_001952_Honey_731_og_23	Chestnut	Chestnut
240131_014604_Honey_1093_og_23	Chestnut	Chestnut
240131_204933_Honey_1256_og_23	Chestnut	Chestnut
230927_093233_Honey_40_og	Citrus	Citrus
230927_233314_Honey_72_og	Citrus	Citrus
240126_161419_Honey_203_og_23	Citrus	Citrus
230930_014606_Honey_141_og	Linden	Linden
230930_032313_Honey_149_og	Linden	Linden
240127_113938_Honey_880_og_23	Linden	Linden
240127_122811_Honey_887_og_23	Linden	Linden
231002_171441_Honey_162_og	Rhododendron	Rhododendron
231003_091851_Honey_176_og	Rhododendron	Rhododendron
240127_082524_Honey_862_og_23	Rhododendron	Rhododendron
240130_124909_Honey_892_og_23	Acacia	Rhododendron
231006_221437_Honey_224_og	Sunflower	Sunflower
240131_182353_Honey_1227_og_23	Sunflower	Sunflower
240131_222641_Honey_1282_og_23	Sunflower	Sunflower
230927_021530_Honey_20_og	Acacia	Taraxacum
240229_101941_Honey_acacia_rappresentativa	Acacia	Acacia
240229_124521_Honey_agrumi_rappresentativo	Citrus	Citrus
240229_110814_Honey_rododendro_rappresentativo	Rhododendron	Rhododendron
240229_133355_Honey_girasole_rappresentativo	Sunflower	Sunflower
240229_142228_Honey_tarassaco_rappresentativo	Taraxacum	Taraxacum

For the annotation of the most intense volatile compounds in each type of monofloral honey, SPME-GC–MS-IMS was applied, and subsequently, the most prominent compounds in each honey were identified and labelled.

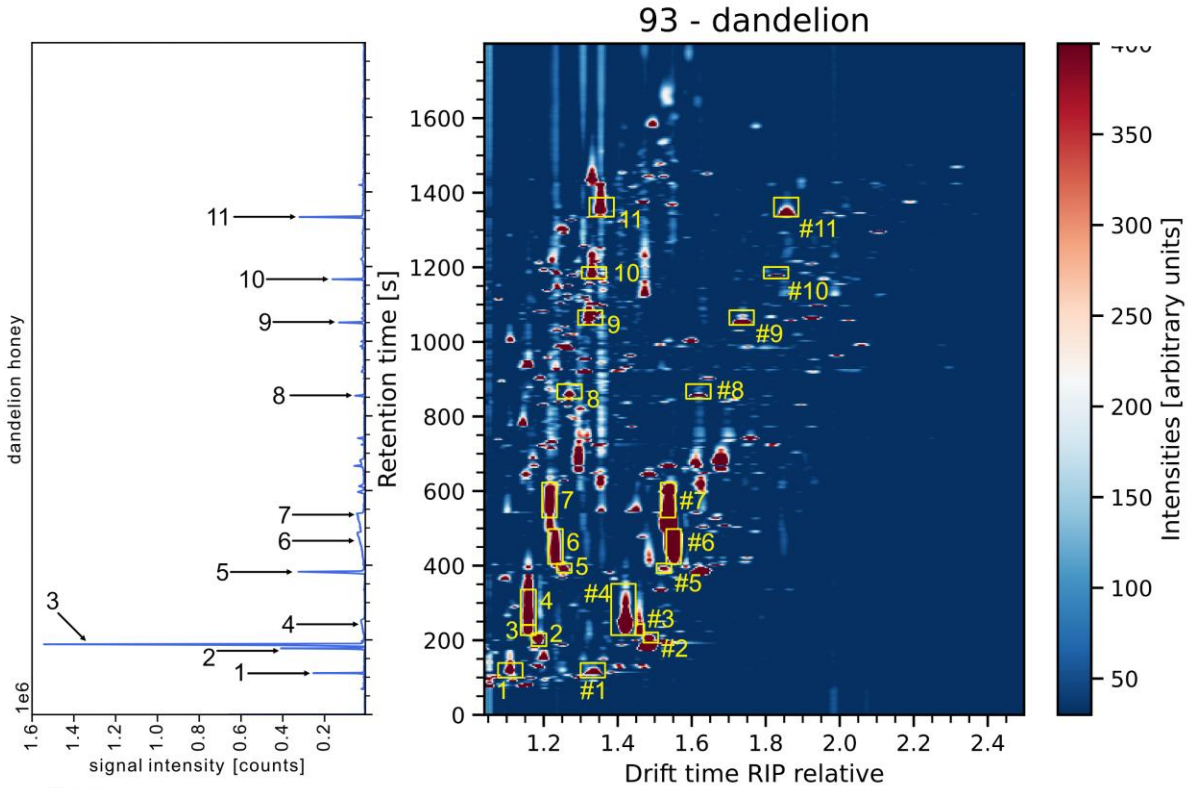


For each sample, 2 g honey and 2 mL of saturated sodium chloride solution were transferred into a 20 mL headspace vial.
Incubation was performed at 60 °C for 15 min.
Extraction time was set to 15 min, desorption time was set to 2 min, and a DVB/C-WR/PDMS 50/30 µm SPME fiber (Restek GmbH, Bad Homburg v. d. Höhe, Germany) was used.
Chromatographic separation was performed with a Nexis™ GC-2030 (Shimadzu Corporation, Kyoto, Japan) and DB-5MS UI capillary column (30 m × 0.25 mm × 0.25 µm; Agilent)
The ion source temperature of the QP2020 NX MSD (Shimadzu Corporation, Kyoto, Japan) was set to 220 °C, the electron ionization energy was 70 eV, the emission current was 150 µA, and the scan range was *m/z* 35 to m/z 400, with a duty cycle of 200 ms.

MS data were analyzed using GCMSsolutions 4.53 (Shimadzu Corporation, Kyoto, Japan) and PARADISe (version 6.0.1, 2025) in combination with NIST/EPA/NIH Mass Spectral Library 23 for substance identification (NIST, Gaithersburg, MD, USA).

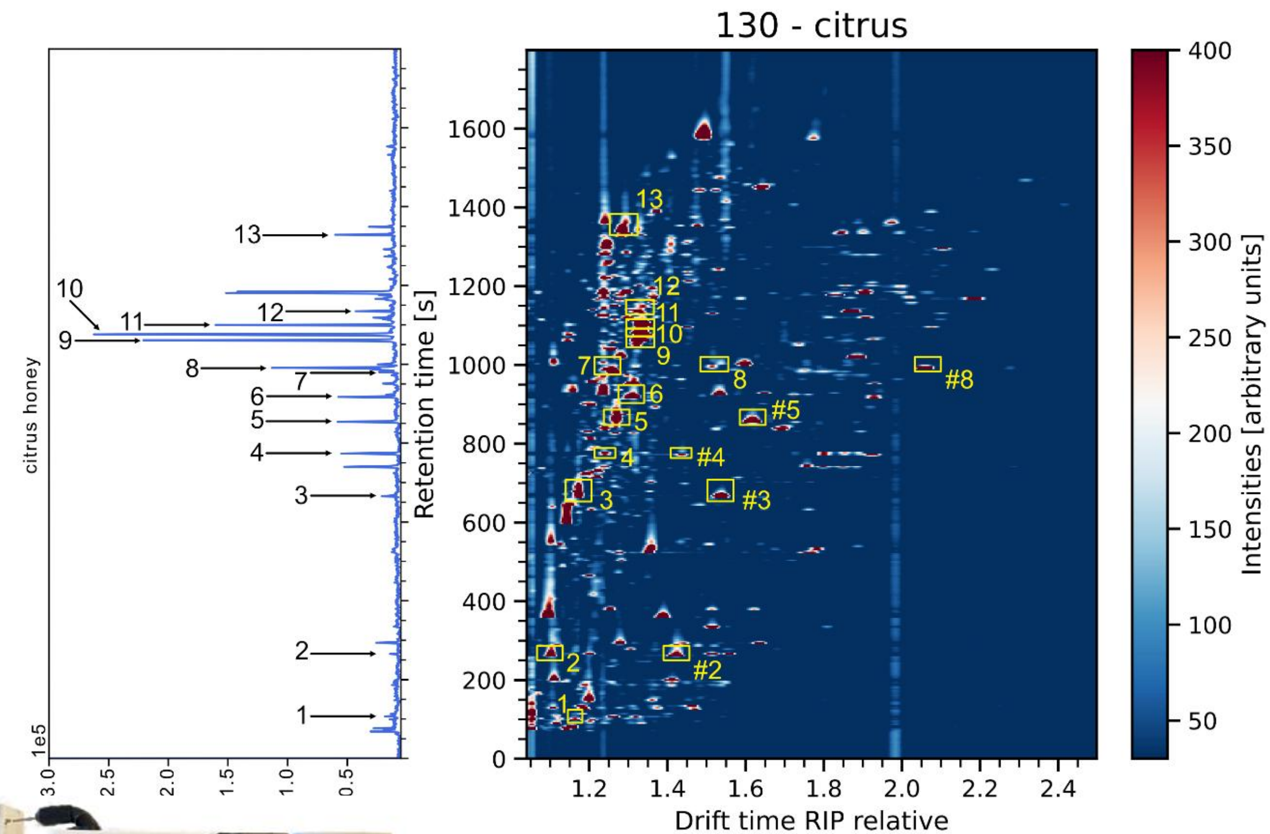
The NIST library was used to annotate the VOCs, with a match ≥90% being considered successful.

DANDELION



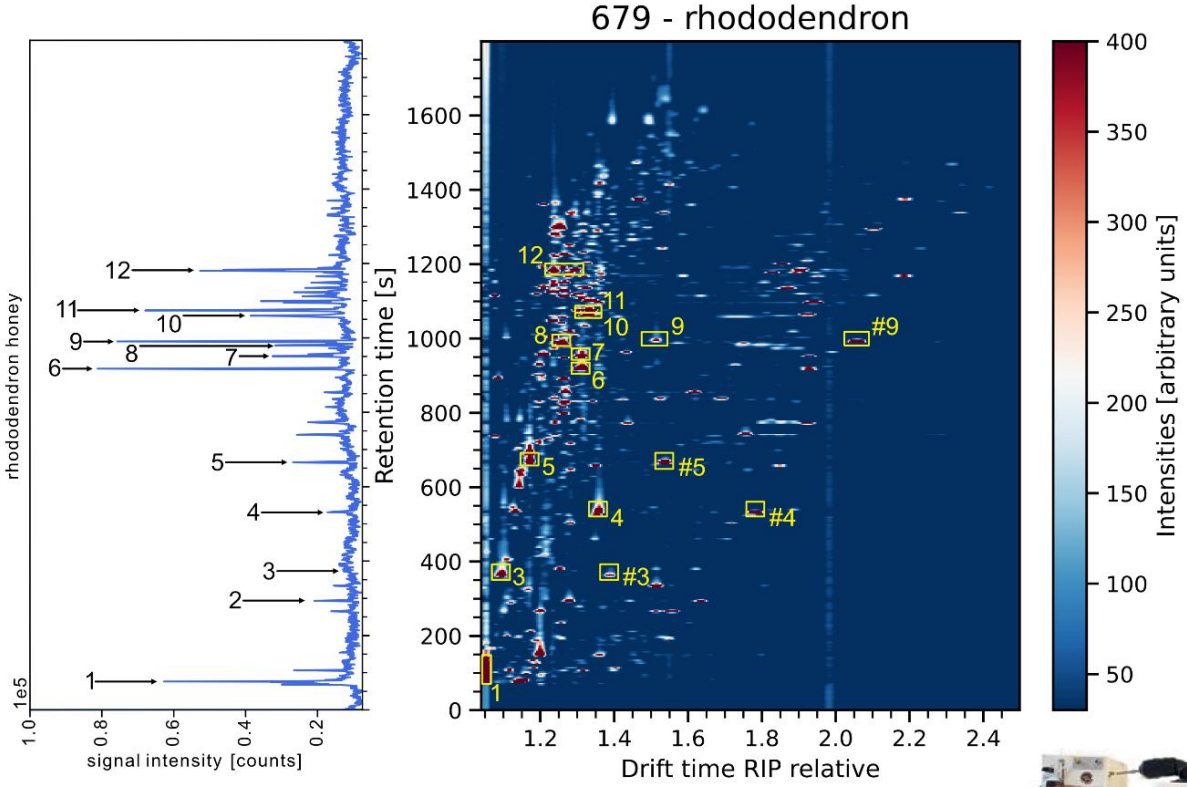
The peaks are: 1) isobutyronitrile; 2) 2-methyl-butanenitrile; 3) 3-methyl-butanenitrile; 4) 2-methylpropanoic acid; 5) 4,4-dimethyl-3-oxopentanenitrile; 6) 3-methylbutanoic acid; 7) 2-methylbutanoic acid; 8) benzene acetaldehyde; 9) benzyl nitrile; 10) ethyl phenylacetate and; 11) 3-hydroxy-4-phenylbutan-2-one.

CITRUS



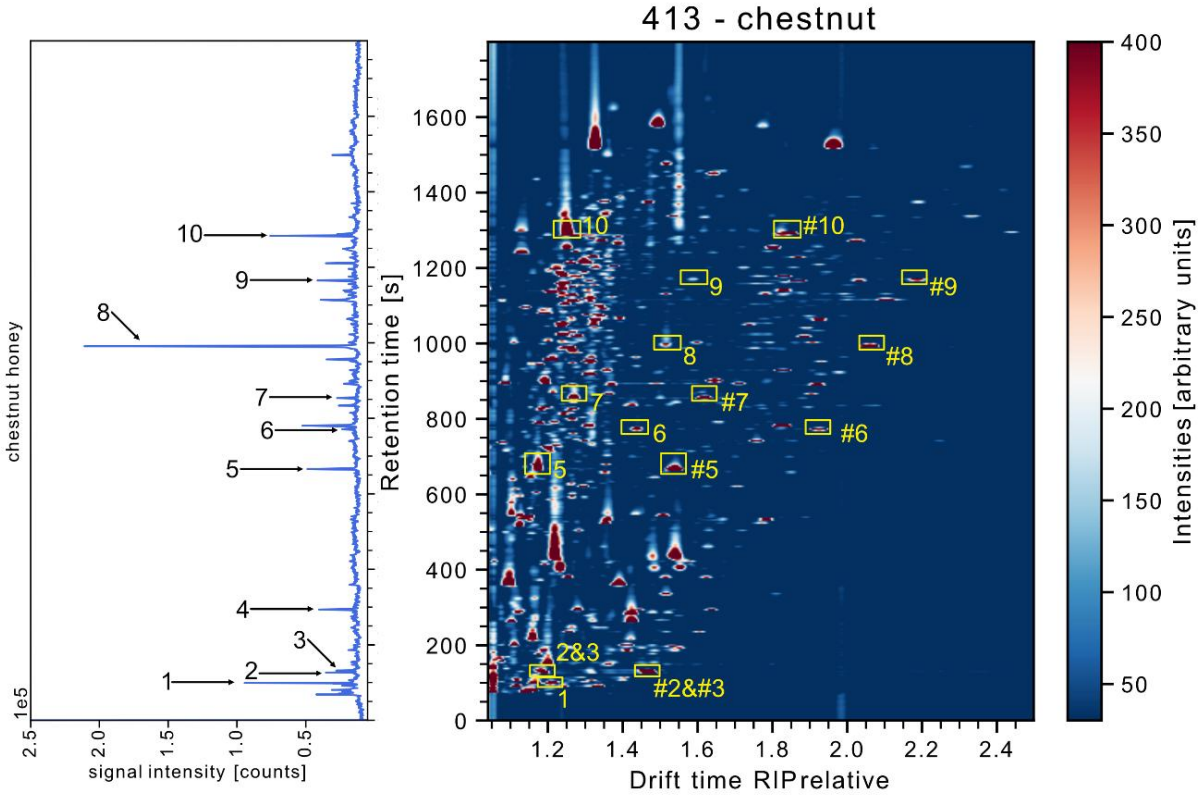
The peaks are: 1) 2-methyl-3-buten-2-ol; 2) 3-hepten-2-one, 3) benzaldehyde, 4) cis-dehydroxy linalool oxide,5) benzene acetaldehyde , 6) cis-linalool oxide , 7) linalool, 8) nonanal, 9) lilac aldehyde a , 10) lilac aldehyde b , 11) lilac aldehyde c, 12) dill ether and 13) methyl anthranilate.

RHODODENDRON



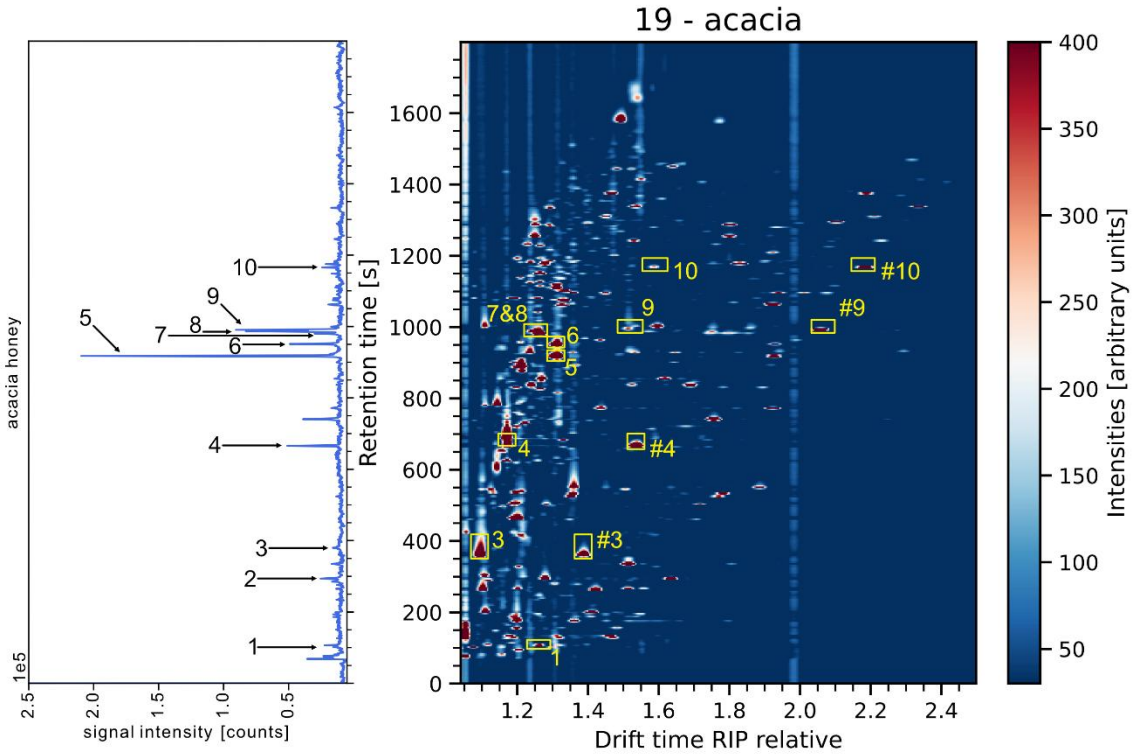
The peaks are 1) ethanol, 2) octane, 3) furfural, 4) heptanal, 5) benzaldehyde, 6) cis-linalool oxide, 7) trans-linalool oxide, 8) linalool, 9) nonanal , lilac aldehyde a, 11) lilac aldehyde b and 12) 1-p-menthen-al isomers. Dimers are marked with a “#”.

CHESTNUT



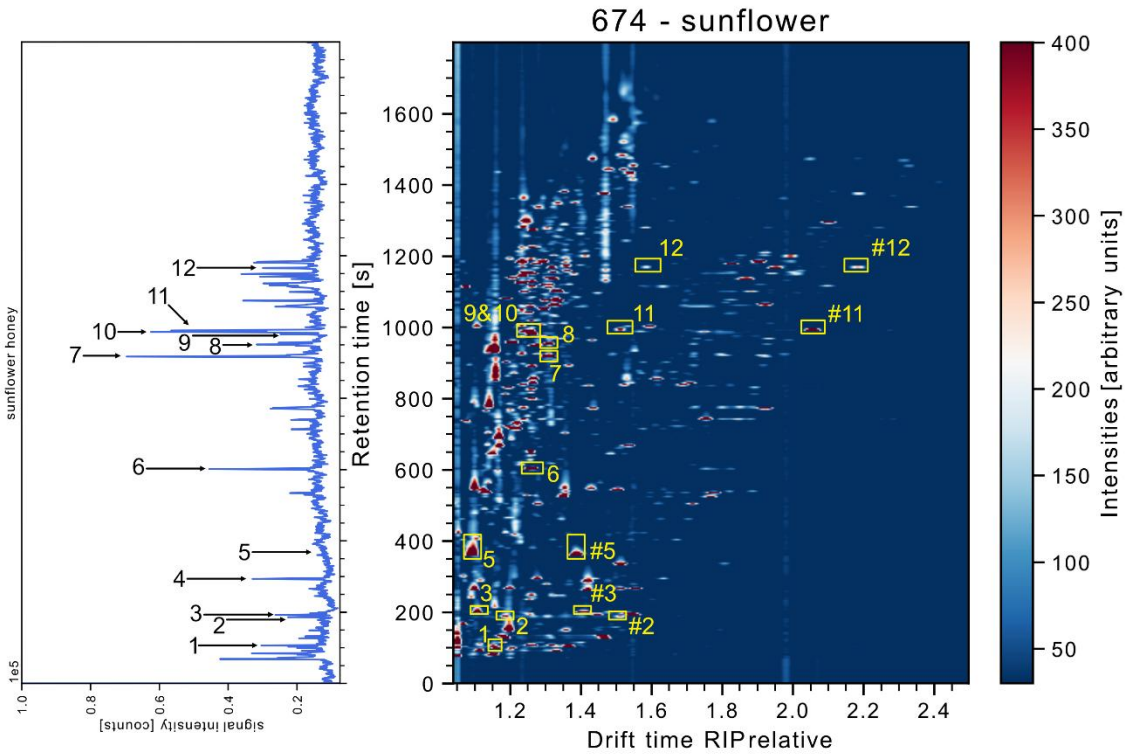
The peaks are 1) 2,3-butanedione, 2) 3-methylbutanal , 3) 2-methylbutanal, 4) octane, 5) benzaldehyde, 6) octanal, 7) benzene acetaldehyde, 8) nonanal, 9) decanal and 10) 2-aminoacetophenone.

ACACIA



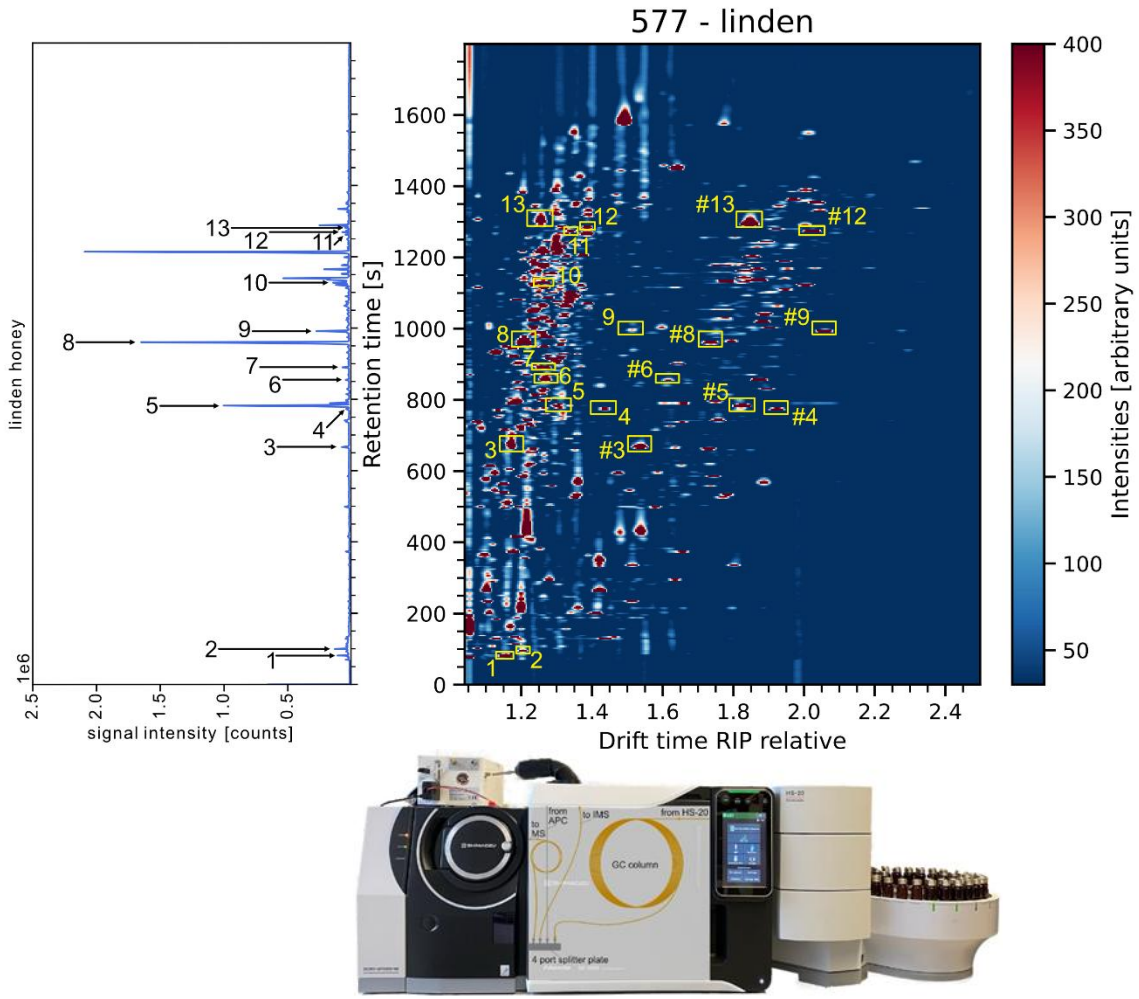
The peaks are 1) 2,3-butanedion, 2) octane, 3) furfural , 4) benzaldehyde, 5) cis-linalool oxide, 6) trans-linalool oxide, 7) linalool, 8) hotrienol, 9) nonanal and 10) decanal.

SUNFLOWER



The peaks are 1) 2-methyl-3-buten-1-ol, 2) 3-methyl-3-buten-1-ol, 3) 3-methyl-1-butanol, 4) octane, 5) furfural, 6) α-pinene , 7) cis-linalool oxide, 8) trans-linalool oxide, 9) linalool,10) hotrienol ,11) nonanal and 12) decanal.

LINDEN



The peaks are 1) acetone, 2) 2,3-butanedione, 3) benzaldehyde, 4) octanal, 5) trans-3(10)-caren-2-ol, 6) benzene acetaldehyde, 7) γ-terpinene , 8) 1-methyl-4-(1-methylethenyl)-benzene, 9) nonanal,10) terpinene-4-ol, 11) thymol, 12) 2-caren-10-al and 13) carvacrol.

Take home message

- We demonstrated the capability of HS-GC-IMS to successfully authenticate the botanical origin of honey
- PLS DA classifier showed good performances
- HS-GC-IMS is a cost-effective source
- HS-GC-IMS allows a minimal sample preparatior
- HS-GC-IMS is commercial available
- Careful late stage validation of the features was attempted but extra efforts are still necessary.

Acknowledgments

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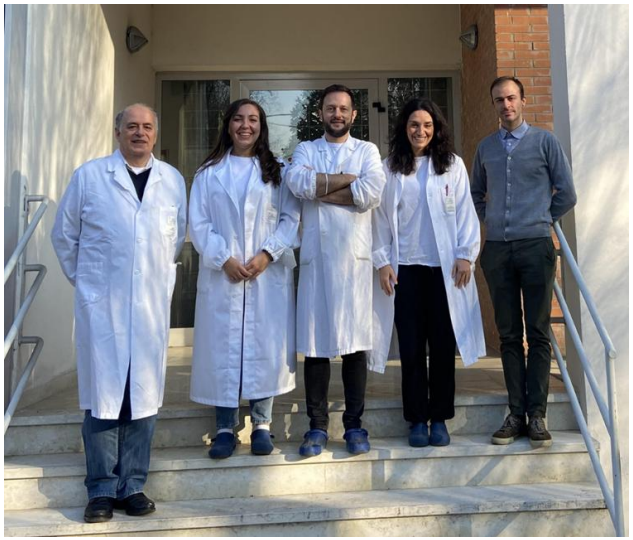

OSSERVATORIO NAZIONALE MIELE

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ISPETTORATO CENTRALE DELLA TUTELA DELLA QUALITA' E REPRESSIONE FRODI DEI PRODOTTI AGROALIMENTARI

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