

HS-GC-IMS authenticates the botanical origin of seven types of Italian monofloral honey, annotating the significant volatile compounds by the novel HS-SPME-GC-IMS-MS prototype

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Introduction Monofloral honey, characterised by nectar predominantly derived from a single plant species, has distinctive properties that increase its commercial value but also make it vulnerable to fraud and mislabelling. New European legislation strengthens controls on botanical and geographical origin. Traditional methods, such as melissopalynology and sensory analysis, are effective but require specialised expertise and lengthy analysis times. This study evaluates HS-GC-IMS combined with machine learning algorithms to authenticate seven types of Italian monofloral honey. The innovative HS-SPME-GC-IMS-MS system integrates chromatographic, ion mobility and mass spectrometric data, improving the characterisation of volatile compounds.

Experimental A total of **136 Italian monofloral honey samples**, representing **seven botanical origins**, were analyzed, and their authenticity was verified through sensory, physicochemical, and melissopalynological analyses. **Volatile organic compounds (VOCs)** were investigated using **HS-GC-IMS** and subsequently identified by **HS-SPME-GC-IMS-MS** (Fig. 2). Spectral data were processed in R through filtering, alignment, and peak detection. PCA and PLS-DA were used for exploratory analysis and classification, respectively. The predictive model, optimized using five-fold cross-validation, was evaluated on an independent set of 29 samples using several statistical performance indicators (accuracy, sensitivity, specificity, Cohen's Kappa, and the area under the receiver operating characteristic curve (AUC-ROC)).

Results HS-GC-IMS analysis revealed distinctive volatile fingerprints among Italian monofloral honeys. **PCA showed clear separation of citrus, chestnut, linden, and sunflower honeys**, whereas rhododendron, acacia, and dandelion exhibited greater overlap (Fig. 3). Nevertheless, PLS-DA successfully discriminated the honey types according to botanical origin (Fig. 3). The classification model achieved excellent performance in **cross-validation (accuracy 99.07%, AUC 96.73%)** and good predictive performance on an **independent test set (accuracy 93.10%, AUC 83.33%)**. The two misclassified samples may reflect contributions from additional nectar and pollen sources, consistent with sensory evaluations.

The annotation of major volatile compounds identified characteristic chemical profiles for each honey type, including nitriles and aromatic compounds in dandelion, aromatic aldehydes and terpenes in citrus and rhododendron, nonanal and long-chain aldehydes in chestnut, linalool-related compounds in acacia, alcohols and monoterpenes in sunflower, and terpenes and aromatic phenols in linden honey. Overall, the combination of HS-GC-IMS and multivariate statistical analysis proved to be a promising tool for the discrimination and authentication of the botanical origin of monofloral honeys.

Conclusions The research demonstrates that HS-GC-IMS, combined with multivariate statistical analysis, is a rapid and cost-effective technique for classifying monofloral honeys and verifying their authenticity. The combined use of GC-IMS-MS improves the identification of volatile compounds, even in cases of co-elution. The two techniques are complementary, and the resulting volatilomic profiles can support traceability, supply-chain controls, and consumer protection, although further validation studies are needed.

Classifier	Classifier evaluation	AUC	Accuracy	Kappa	Sensitivity	Specificity
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%

Tab. 1: classification performance indicators for the evaluations of the partial least squared discriminant analysis (PLS-DA) classifier.

Reference <https://doi.org/10.1016/j.microc.2026.117526>.

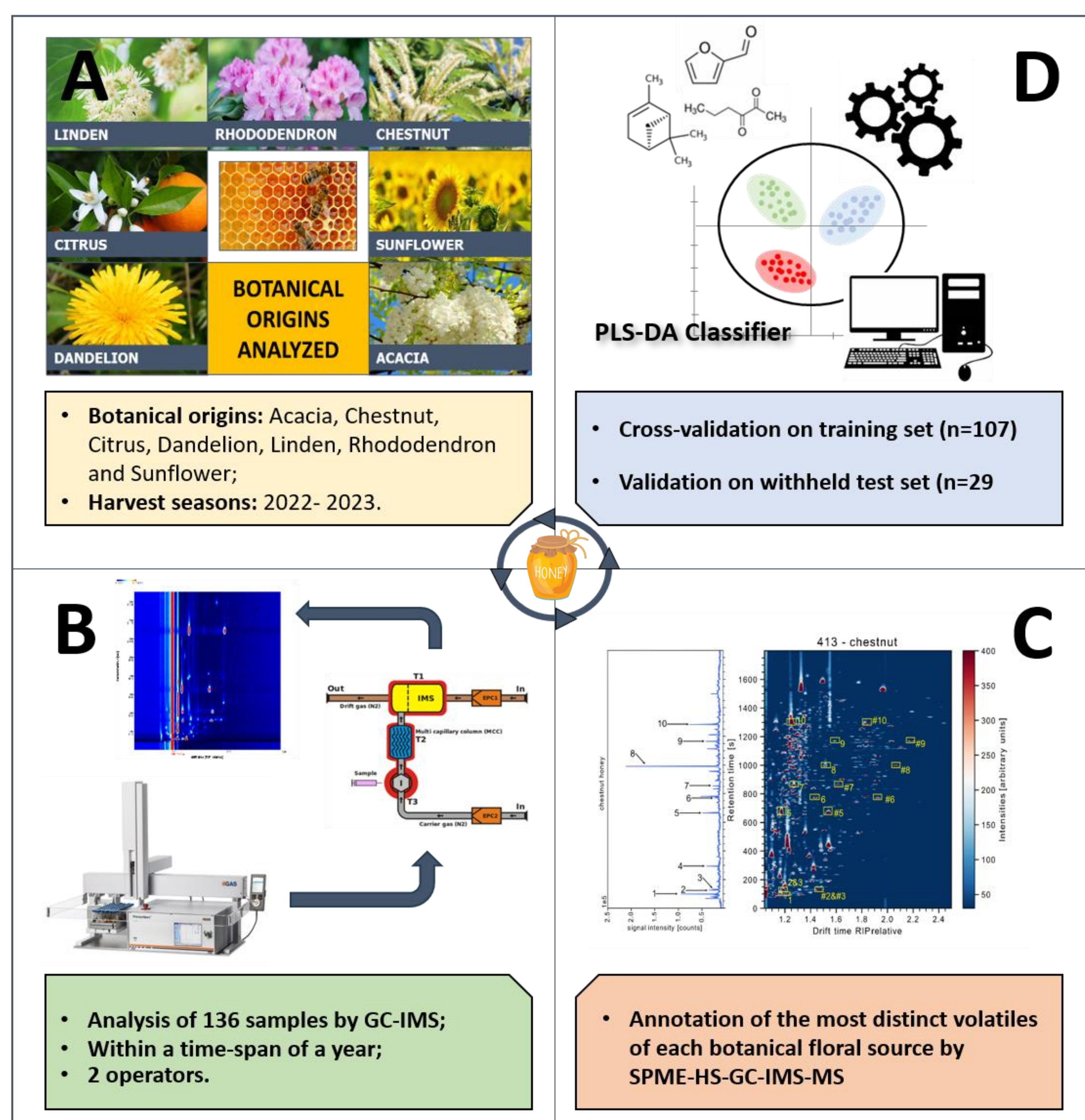


Fig. 1: procedural framework of the study. **A)** A collection of 136 honey samples from seven different botanical origins (acacia, chestnut, citrus, dandelion, linden, rhododendron, sunflower) were collected during 2022 and 2023 in Italy. **B)** The honeys were analyzed by head space gas chromatography ion mobility spectrometry (HS-GC-IMS). 2D chromatograms were generated and separation of volatile compounds was achieved. **C)** Annotation of the most distinct volatiles of each botanical floral source by headspace solid-phase micro-extraction gas chromatography ion mobility spectrometry mass spectrometry (SPME-HS-GC-IMS-MS). **D)** The resultant dataset was preprocessed and, after graphical evaluation of the discrimination capabilities of the HS-GC-IMS for authenticating the monofloral honey, used to build a partial least squared discriminant analysis (PLS-DA).

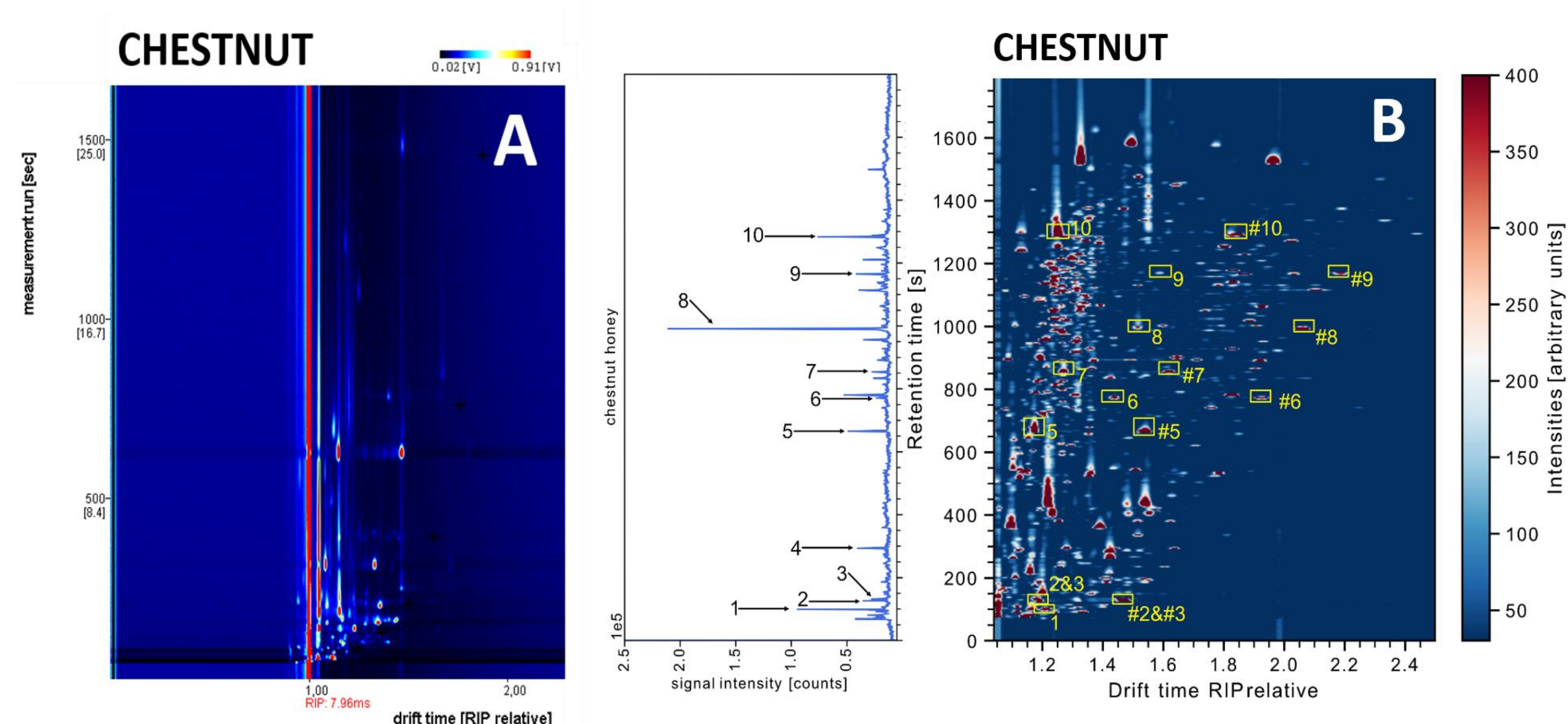


Fig. 2: **A)** Representative head space gas chromatography ion mobility spectra (HS-GC-IMS) of chestnut honey; **B)** Exemplary visualization of a chestnut honey and the simultaneous sample data of MS spectrum (left) and IMS spectrum (right) with selected peaks. The peaks are 1) 2,3-butanedione, 2) 3-methylbutanal, 3) 2-methylbutanal, 4) octane, 5) benzaldehyde, 6) octanal, 7) benzene acetaldehyde, 8) nonanal, 9) decanal and 10) 2-aminoacetophenone. Dimers are marked with a "#".

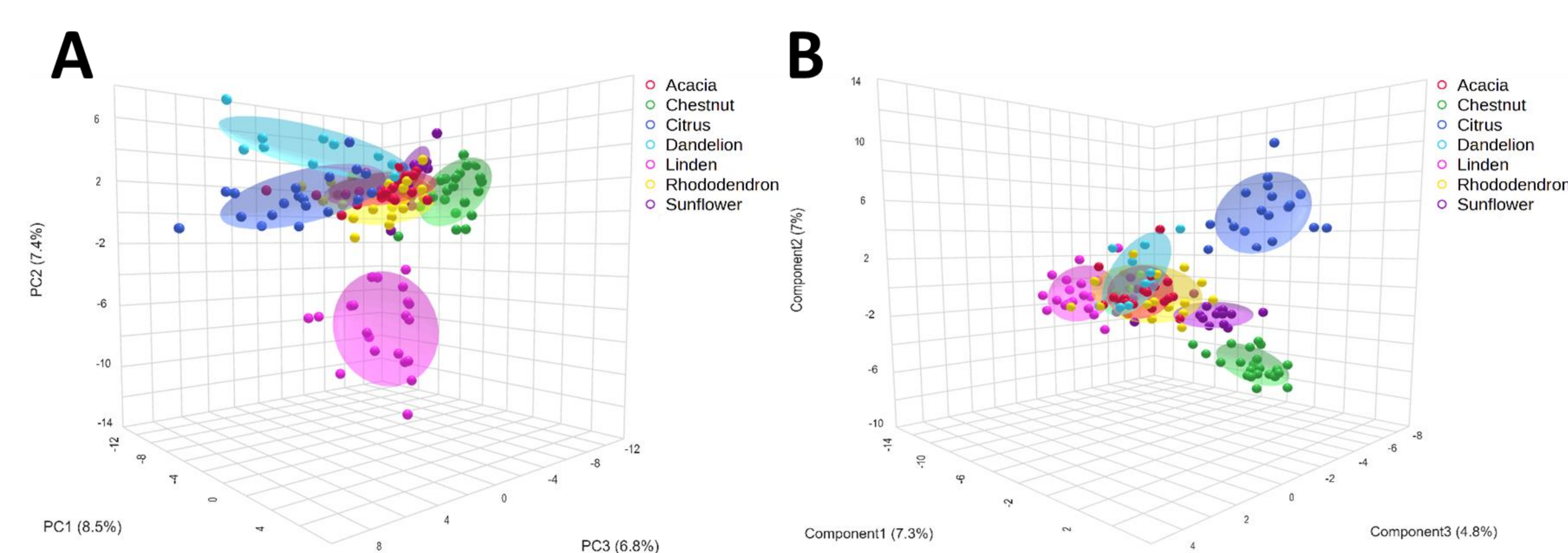


Fig. 3: **A)** principal component analysis (PCA); **B)** partial least squared discriminant analysis (PLS-DA) scores plots. In both graphical representations, the tendency of the volatile fingerprints of the seven types of honey to cluster can be observed.