Botanical discrimination of well-known Greek honey varieties using UPLC-QToF/MS targeted and untargeted metabolomics.

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Introduction

Honey is a natural sweetener and the most important product of beekeeping by ancient times. Moreover, its consumption is correlated to many beneficial properties for human health due to its antioxidant capacity. Thus, due to its high demand and production cost, it is subjected to fraudulent practices. Phenolic compounds, which can be estimated as the most valuable nutritive constituents of honey samples, can be used as authenticity markers. This can be attributed to the fact that the phenolic content of honey is greatly affected by geographical and botanical origin. So, the evaluation and verification of honey authenticity are of paramount importance for the producers, consumers and regulatory bodies.

Materials

In this study, a validated UPLC-ESI-QTOF method was used to detect simultaneously many compounds and identify new markers for the differentiation of samples according to their origin. Honey extracts were eluted with a 20 min gradient program. An Acclaim RSLC C18 column (2.1 × 100 mm, 2.2 μm) was used. The samples were analyzed in negative mode. A generic extraction protocol with ethyl acetate as extractant was used to apply target, suspect and non-target screening approaches. This method was applied to over 100 Greek honey samples from 5 different botanical origins. The target and suspect screening approaches were performed using Data analysis 4.4 and TASQ 1.4, while the non-target screening was performed with Metaboscape 3.0 (Bruker Daltonics).

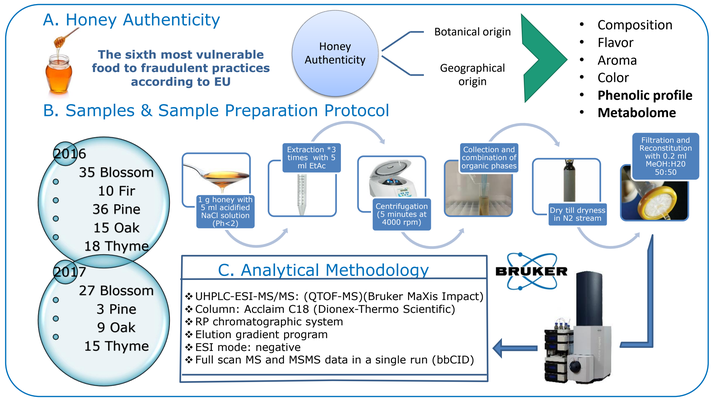
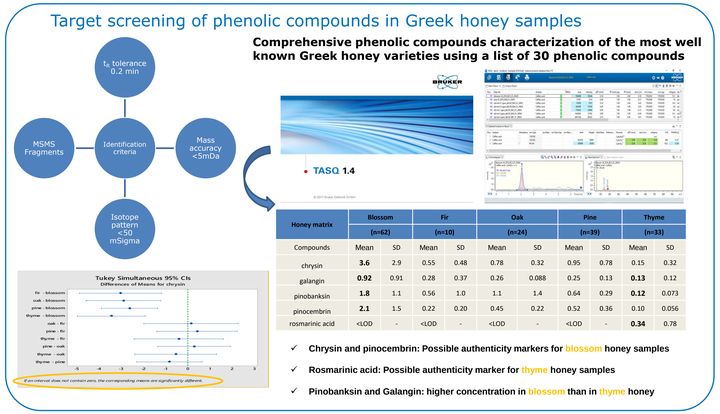


Fig. 1: A. Honey authenticity approach. B. Number of samples and sample preparation protocol. C. Chromatographic and MS parameters used for samples analysis.



Honey matrix	Blossom (n=62)		Fir (n=10)		Oak (n=24)		Pine (n=39)		Thyme (n=33)	
Compounds	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
chrysin	**3.6**	2.9	0.55	0.48	0.78	0.32	0.95	0.78	0.15	0.32
galangin	**0.92**	0.91	0.28	0.37	0.26	0.088	0.25	0.13	**0.13**	0.12
pinobanksin	**1.8**	1.1	0.56	1.0	1.1	1.4	0.64	0.29	**0.12**	0.073
pinocembrin	**2.1**	1.5	0.22	0.20	0.45	0.22	0.52	0.36	0.10	0.056
rosmarinic acid	<LOD	-	<LOD	-	<LOD	-	<LOD	-	**0.34**	0.78

- ✓ Chrysin and pinocembrin: Possible authenticity markers for blossom honey samples
- ✓ Rosmarinic acid: Possible authenticity marker for thyme honey samples
- ✓ Pinobanksin and Galangin: higher concentration in blossom than in thyme honey

Fig. 2: Target screening of phenolic compounds in honey samples using TASQ 1.4 and suggestion of possible markers for honey authenticity assessment.

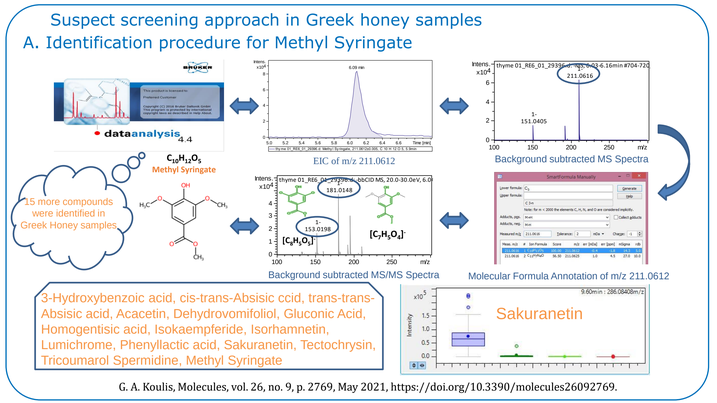


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Fig. 3: Suspect screening approach for the characterization of Greek honey samples.

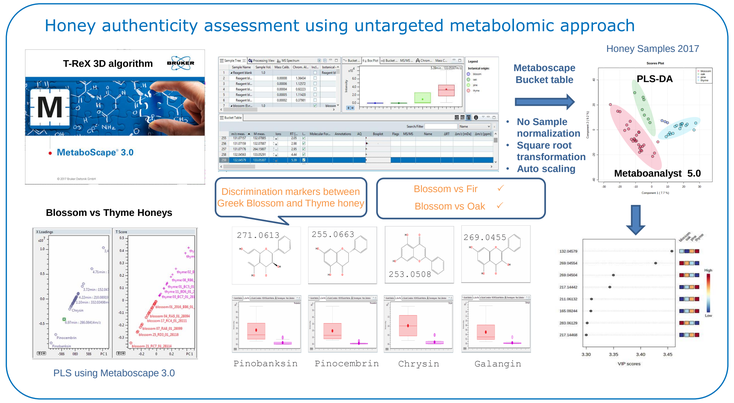

Fig. 4: Untargeted metabolomic approach for the botanical discrimination of Greek honey samples using Metaboscape 3.0.

Results

A comprehensive characterization of bioactive content of the most well-known Greek honey varieties was achieved through target and suspect screening. Regarding the target screening, many phenolic compounds have been detected and quantified in each category, revealing important potential markers for honey authenticity assessment as Chrysin and Pinocembrin for blossom honey and Rosmarinic acid for thyme honey.

Furthermore, 15 phenolic and non-phenolic compounds with significant health benefits were identified through suspect screening, providing a wealth of data for honey bioactive content characterization. Although suspect screening does not show any potential marker, Sakuranetin proved to be higher in blossom samples.

Finally, through the non-target screening using Metaboscape 3.0 and PLS and PLS-DA statistical analysis, authenticity markers were revealed for the discrimination of honey samples according to their botanical origin. According to the PLS model, discrimination between blossom and thyme honey was achieved with the most important variables: Chrysin, Pinocembrin, Galangin and Pinobanksin. Moreover, blossom were differentiated from oak honey with Salicylic acid and 3,4 dihydroxybenzoic acid as main contributors and from fir honey with Chrysin, Pinobanksin and Sakuranetin as the most important variables. The PLS-DA model for 2017 honey samples has distributed them into four distinct groups with great predictive ability revealing important markers.

Conclusions

- Comprehensive bioactive content characterization was achieved through target and suspect screening revealing important markers for honey authenticity assessment.
- Chrysin and Pinocembrin higher in blossom honey , While Rosmarinic acid in thyme honey.
- 15 important compounds were identified using suspect screening approach contributing to the comprehensive characterization of the bioactive content of the most important Greek honey varieties.
- Authenticity markers were identified using metaboscape 3.0 and chemometrics for the botanical origin discrimination.

QTOF / Foodomics

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