ASSESSING THE CONTENT OF PHYTOCHEMICALS IN HONEY SAMPLES: POLYPHENOLS AS BIO-INDICATORS OF THEIR BOTANICAL ORIGIN

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HONEY

Honey is a natural food product well known for its high nutritional value that contains phytochemicals with highly antimicrobial and antioxidant capacities [1,2].

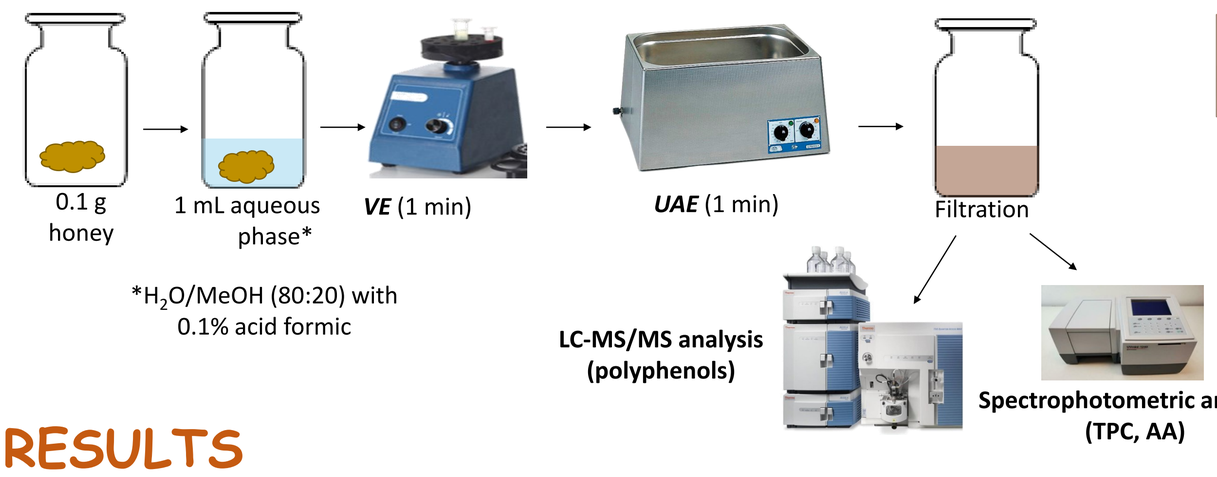
The ***main goal of this work*** is the ***development of an analytical method to obtain the polyphenolic profile of honeys*** from different varieties. ***Miniaturized vortex (VE)*** and ***ultrasound assisted extraction (UAE)*** were employed and the analysis of 40 target polyphenols was carried out by liquid chromatography-tandem mass spectrometry (***LC-MS/MS***). Total polyphenolic content (***TPC***) and antioxidant activity (***AA***) were also evaluated. Finally, analysis of variance (***ANOVA***) and principal component analysis (***PCA***) were employed to ***obtain models that allow classifying the different honeys according to their origins***.

SAMPLING

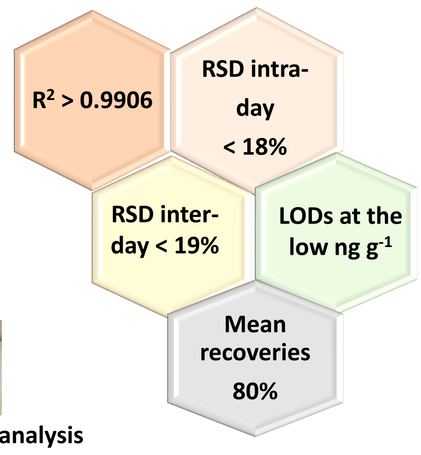
91 honey samples from Galicia (NW Spain) were collected from the flowering season of 2018 and 2019

Varieties Honeydew (HD), blackberry (BL), heather (HE), chestnut (CN), eucalyptus (EU) and multi-floral (MF). In addition, samples from unknown origin (UnK) were also analyzed.

MINIATURIZED SAMPLE PREPARATION



VE-UAE-LC-MS/MS VALIDATION



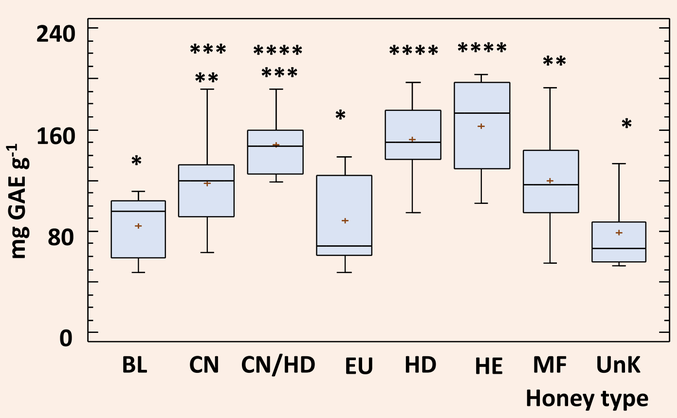
40 target polyphenols

Gallic acid; 2,4-6-trihydrobenzoic acid; 2-4-dihydroxybenzoic acid; 3-4-dihydroxybenzoic acid; cafftaric acid; 2-5-dihydroxybenzoic acid; Procyanidine B1, B2, A1, A2, C1; 2-6-dihydroxybenzoic acid; 3-5-dihydroxybenzoic acid; Catechin; 4-HydroxyBenzaldehyde; 3-hydroxybenzoic acid; Chlorogenic Acid; 3-4-dimetoxybenzoic acid; Caffeic Acid; Epicatechin; GallocatechinGallate; p-coumaric acid; EpicatechinGallate; 7-HydroxyCoumarin; CatechinGallate; Orientine; 3-4-DimethoxyBenzaldehyde; 4-MethoxyBenzaldehyde; Quercetin-3-Glucuronide; Quercetin-3-rutinoside; Quercetin-3-Glucoside; Myricetin; Quercetine; Kaempferol; Apigenin; Chrysin; Trans-Ferulic Acid; 4-hydroxyphenylacetic acid; Vanillic acid; 4-hydroxybenzoic acid

RESULTS

One-way ANOVA was performed to assess statistical differences between honeys botanical origin attending to their bioactive properties: total polyphenolic content (TPC) and antioxidant activity (AA).

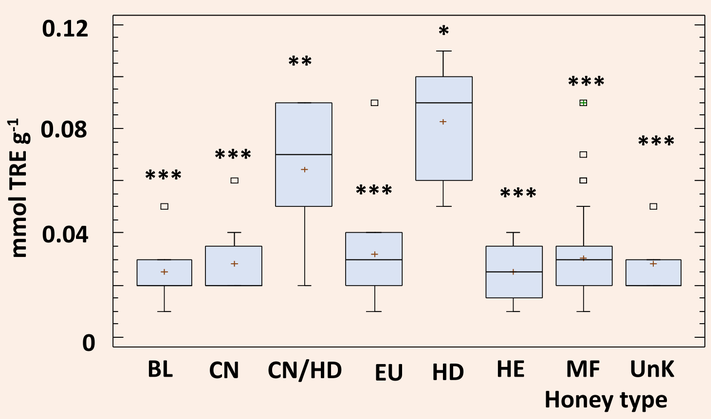
Total polyphenolic content (TPC)



* p-value between groups was <0.05. Different symbols indicate statistically significant differentes at p < 0.05.

Attending to TPC results, 4 different homogeneous groups could be obtained: (i)BL, EU, UnK, (ii) CN, MF, (iii) CN, CN/HD, (iv) CN/HD, HD, HE.

Antioxidant activity (AA)

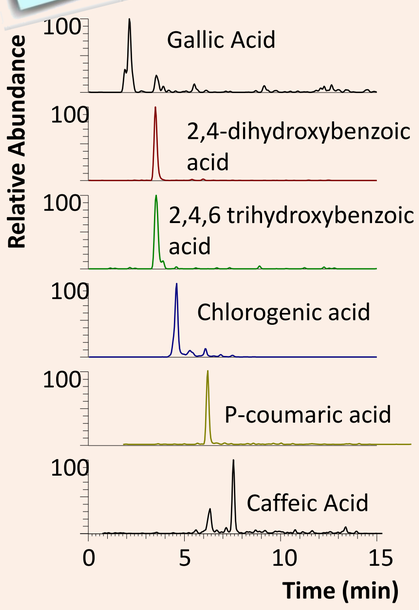


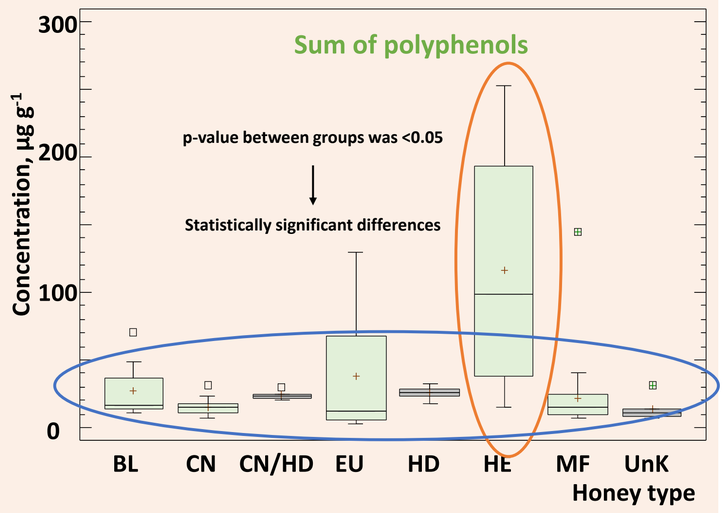
* p-value between groups was <0.05. Different symbols indicate statistically significant differentes at p < 0.05.

Attending to AA results, 3 groups statistically different were obtained: (i) HD, (ii) CN/HD, (iii) BL, CN, EU, HE, MF, UnK.

After validation, the VE-UAE-LC-MS/MS method was applied to the 91 honey samples to quantify 40 target polyphenols.

Real sample chromatogram





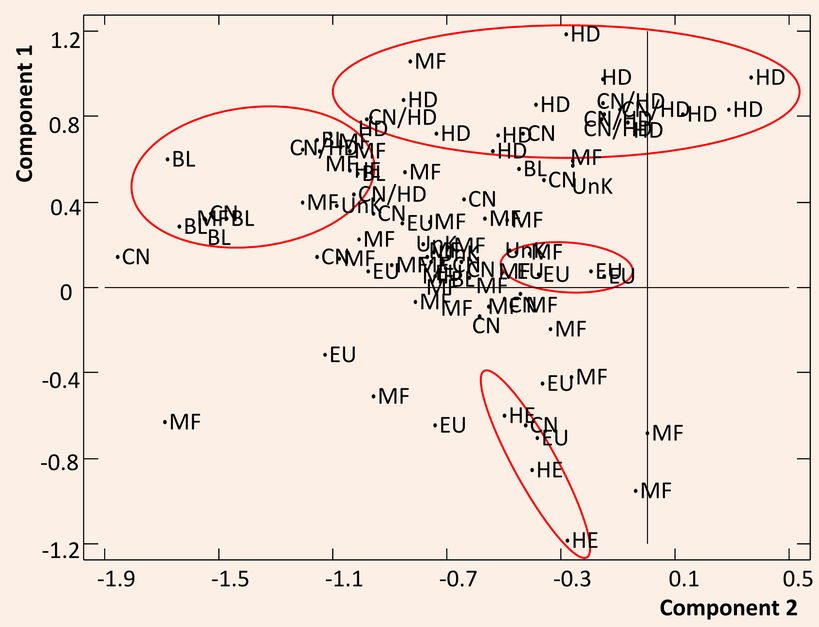
25 out of the 40 target polyphenols were found in the analyzed samples.

HE variety showed the highest concentration of polyphenols, reaching levels of 250 µg g^{-1}.

Considering the sum of polyphenols only two groups were statistically different:

(i) HE; (ii) the rest of honey varieties

TENTATIVE CLASSIFICATION OF THE HONEY SAMPLES BY PCA



- PCA was applied including the 91 analyzed samples and 25 variables (the detected polyphenols by LC-MS/MS analysis).
- Two components were enough to explain more tan 90% of variance.
- PC1 was mainly influenced by p-coumaric acid, whereas PC2 for other polyphenolic derived acids

CONCLUSIONS

- A miniaturized and environmentally-friendly method based on VE-UAE-LC-MS/MS employing aqueous solvent was successfully validated to evaluate the polyphenolic profile of honey samples.
- Results revealed that 25 different polyphenols were detected in the analyzed samples, reaching up to hundreds of µg g^{-1}.
- ANOVA and PCA based on the results from TPC, AA and sum of polyphenols determination showed significant differences depending on the honey variety.
- This study demonstrates that the combination of a miniaturized UAE based method, LC-MS/MS measurements and PCA tool, is a suitable strategy to investigate the botanical authentication of honey.

Acknowledgements & references

This research was supported by project GO FEADER 2018/054B (Xunta de Galicia). The authors belong to the National Network for the Innovation in miniaturized sample preparation techniques, RED2018-102522-T (Ministry of Science, Innovation and Universities, Spain), and to the Galician Competitive Research Groups IN607B 2019/13 and ED431 2020/06 (Xunta de Galicia). This study is based upon work from the Sample Preparation Study Group and Network, supported by the Division of Analytical Chemistry of the European Chemical Society. All these programmes are co-funded by FEDER (EU).

Authors acknowledge the Regulator Council of Galician Honey (IXP, Mel de Galicia) for supplying the samples.

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RED NACIONAL PARA LA INNOVACIÓN EN LAS TÉCNICAS DE TRATAMIENTO DE MUESTRAS MINIATURIZADAS