Potential of trapped-ion-mobility UHPLC-QTOF in food authenticity studies: characterization of co-eluting secoiridoids isomers found in Greek extra virgin olive oil

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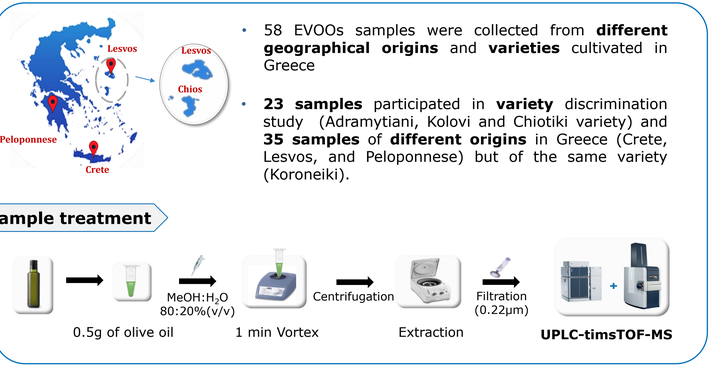
Introduction

Extra virgin olive oil (EVOO) has been declared as an integral part of a Mediterranean diet for its high nutritional, sensory and health protecting properties. Olive oil mainly consists of glycerides and fatty acids that represent more than 98% of the total oil weight. However, many of its characteristic properties result from the small proportion of ~ 2% by weight, which contains several phenolic compounds. Phenols and phenolic derivatives are classified among the most important constituents in olive oil, as their presence indicates an increasing potential for health protection. The European legislative framework (EU 432/2012), highlights the contribution of polyphenol rich EVOOs to "the protection of blood lipids from oxidative stress". Special attention has been paid to secoiridoids due to the high concentrations detected.

Methods

In the present study, a novel methodology based on Trapped Ion Mobility Spectrometry (TIMS) was applied. EVOO extracts were eluted with a 15 min gradient, including a flow gradient (0.4-0.6 mL/min), on an UHPLC using a C18 (2.1 x 100 mm, 1.8 µm) column, with acidified water and methanol. The UHPLC was coupled to a timsTOF Pro (Bruker Daltonics) and analyzed in negative and positive mode using PASEF for optimal MS/MS performance. EVOOs of different variety and geographical origin were analyzed in order to study differentiations among the samples; the aim being to globally profile the compounds. Data was acquired and processed using MetaboScape® 4.0 (Bruker Daltonics) and the T-ReX 4D algorithm. For statistical evaluation PCA and PLS-DA were used.



- 58 EVOOs samples were collected from **different geographical origins** and **varieties** cultivated in Greece
- **23 samples** participated in **variety** discrimination study (Adramytiani, Kolovi and Chiotiki variety) and **35 samples** of **different origins** in Greece (Crete, Lesvos, and Peloponnese) but of the same variety (Koroneiki).

Fig. 1: EVOOs sampling of different varietal and origin background, processed with a simple LLE protocol and analyzed in timsTOF.

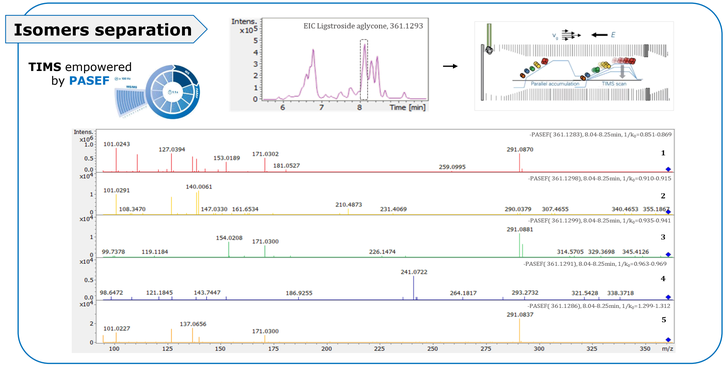


Fig. 2: TIMS contribution to isomers separation and PASEF enhancement to secoiridoids structure elucidation, through high-quality MS/MS spectra acquisition.

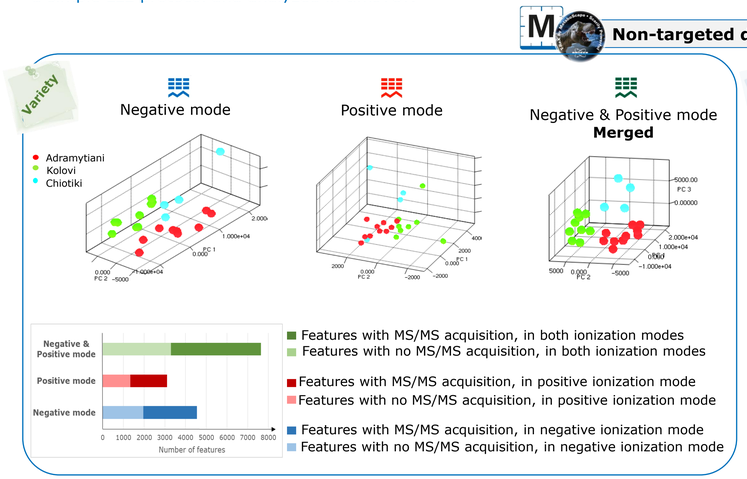


Fig. 3: EVOOs varietal discrimination using both ionization-runs data, merged into a single dataset for better clustering in PCA and acquisition of more MS/MS data.

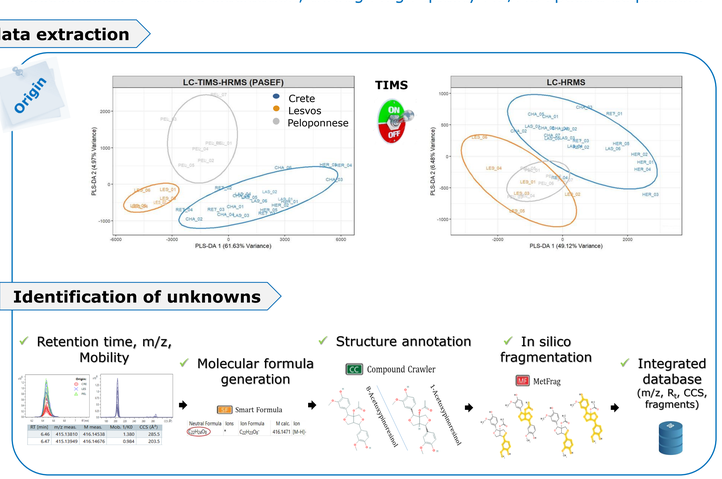


Fig. 4: Comparison of LC-TIMS-HRMS to LC-HRMS methodology in terms of EVOOs geographical origin discrimination and schematic workflow for the identification of VIP compounds.

Results

In the present study we were particularly interested in the separation and identification of secoiridoids and their isomers, crucial in EVOOs health claim. Exploiting the capability of mobility dimension and the additional information of collision cross section (CCS), isomer separation was achieved. More specifically, isomers that could not be discriminated using HRMS-based workflows, due to identical chromatographic and spectral profile were successfully separated and identified for the first time, to the best of our knowledge. Further investigation was conducted through PASEF (Parallel Accumulation Serial Fragmentation) data dependent acquisition that enabled in-depth structure elucidation of isomers. Noteworthy differentiations, regarding the profile of isomeric secoiridoids were highlighted in samples of different variety, providing strong indication that isomers could be used as potential authenticity markers. Finally, an integrated workflow for the identification of unknowns, comprising all information retrieved trough LC-TIMS-MS applied methodology, was also introduced.

Conclusions

- The additional dimension of analysis provided by TIMS led to the separation and identification of isomeric compounds.
- High-quality spectra obtained using PASEF acquisition mode enabled an in-depth structure investigation of secoiridoids.
- Negative and positive mode complementarity was evaluated through variety discrimination study, resulting in better data clustering and acquisition of more MS/MS data.
- LC-TIMS-HRMS applicability in authenticity studies was highlighted, with isomers indicated as potential markers and further studied through an integrated identification workflow.

timsTOF / Foodomics