Application of metabolomics methods on LC/GQ-QTOF data to discriminate extra virgin olive oils from different Protected Designations of Origin

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Introduction

The increasing popularity of extra virgin olive oil (EVOO) and the increasing problem of food fraud have provided the need for quality and authenticity control. Typical problems are mislabeling of protected designation of origin (PDO) or edible oil adulteration. Implementation of protected designations of origin (PDOs) and protected geographical indications (PGIs) is one of the most prominent differentiation strategies used in olive oil market. They are often perceived as valuable tools that promote specific attributes of the oil linked to its geographical provenance. Minor compounds of extra virgin olive oil, such as phenolic and triterpenic compounds, sterols and tocopherols, are highly influenced by agro-technological practices and can be used for olive oil authentication.

Methods

In this study 126 oil samples from 6 Mediterranean PDOs were analyzed by LC-MS and GC-MS combined to statistical methods. The 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 acetonitrile. The column oven temperature was 40° C. The derivatized extracts were injected in GC, using a BR-5 column with a 50 min T gradient from 150 to 320°C (4°C/min rate). Both systems were coupled to a Compact™ QTOF MS (Bruker) by an ESI and an APCI interface for LC and a GC-APCI source for GC.

(126 samples)		
Country	PDO/PGI	N
SPAIN	Priego de Córdoba	25
	Baena	19
MOROCCO	Meknès	22
	Ouezzane	25
ITALY	Toscano	20
GREECE	Kalamata	15

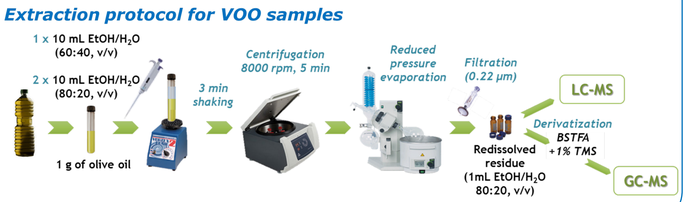


Fig. 1: 126 samples were collected from 6 different regions and processed with a simple LLE protocol.

LC method

- **Bruker C18 column** (2.1x 100 mm, 1.8 µm)
- **Mobile Phase Gradient**
  - A = Water + 0.5 % AcH
  - B = ACN + 0.5 % AcH
- **Flow Gradient:** 0.4-0.6 mL/min
- **Temperature:** 40°C

GC method

- **BR-5 column** (30 m x 0.25 mm i.d., 0.25 µm)
- **Column Flow:** 1 mL/min He
- **Injector T:** 250°C
- **Transfer Line T:** 290 °C
- **Injection mode:** Split 1:25

Fig. 2: LC and GC methods to analyze the olive oil extracts

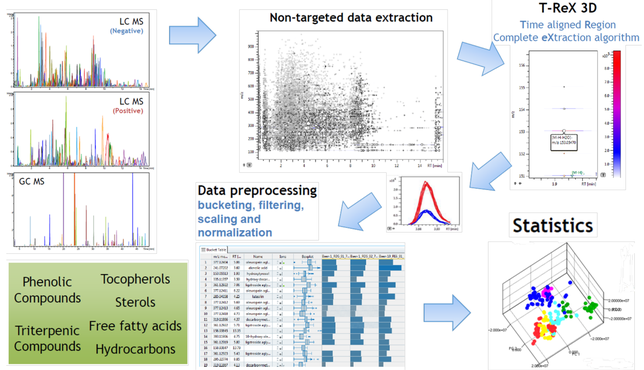
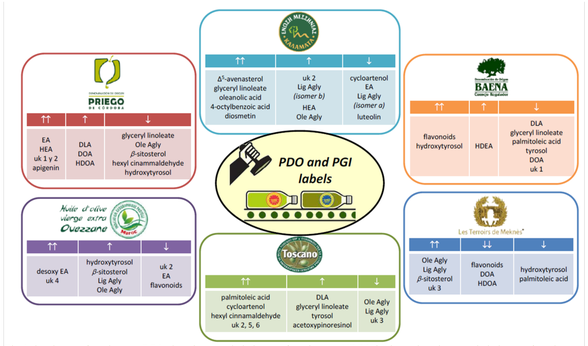


Fig. 3: Workflow for identification of markers for with MetaboScape. After feature extraction and application of T-ReX 3D a bucket table was created. Based on the resulting bucket table PCA and PLS-DA were applied and models for GI markers built.



Ole Agly: oleuropein aglycone; DOA: decarboxymethyl oleuropein aglycone; HDOA: hydroxy decarboxymethyl oleuropein aglycone; Lig Agly: ligstroside aglycone; DLA: decarboxymethyl ligstroside aglycone; EA: elenolic acid; HEA: hydroxy elenolic acid; HDEA: hydroxy decarboxymethyl elenolic acid; uk: unknown;↑↑ very high, ↑ high and ↓ low content

Fig. 4: Summary of the most influential markers found for each geographical indication (GI) combining the information obtained by all statistical models built with the data from all the used platforms.

Results

Data acquired with all the platforms was processed with MetaboScape 3.0 (Bruker), which automatically extracts and combines isotopes, adducts and fragments belonging to the same compound into one feature. The resulting bucket table was used for statistical analysis. Non-targeted and targeted approaches were used to offer maximum coverage of the olive oil metabolome's chemical space in a first step, and the possible validation of the identified markers afterwards. Statistical analysis (PCA, PLS-DA) led to a noticeable discrimination among the six evaluated PDOs considering the data coming from LC-MS and GC-MS. Several compounds such as elenolic acid, luteolin, oleuropein and ligstroside aglycones, and some other tentatively identified substances, were identified as possible PDOs distinctive markers. They enabled the discrimination among different PDOs. The combined use of non-targeted and targeted approaches enhanced the outcome of the study. GC-APCI-Q TOF preserves the pseudo-molecular ion information, which is a great advantage over the "classical" GC-EI-MS systems and facilitates the identification of unknown markers.

Conclusions

- The power of different sophisticated methodologies (covering VOO minor fractions) together with statistics to classify oils from diverse origins had been checked.
- Different 2-class models have been built with the aim of pointing out PDO-markers.
- The different polarities and platforms logically drove to diverse makers, taking advantage of their complementarity and, consequently, enriching the outcomes of the project.
- In order to model the seasonal variability too, it would be necessary to enrich the created models by using oils coming from different seasons.

QTOF / Foodomics