



LABORATORY REPORT

SUBJECT: Qualitative and quantitative analysis of polyphenols in olive oil samples

DATE: 07/05/2018

SAMPLES	
NAME	INFORMATION
OLE_1375	Olive Oil sample

ANALYSIS

- A. Extraction of biophenols
- B. HPLC-DAD Analysis – profiling
- C. Qualitative and quantitative analysis of certain biophenols
- D. Qualitative analysis of the hydrolyzed olive oil extract

OLIVE OIL SAMPLES INFORMATION

SAMPLE CODE	OLE_1375
Information	D. MOURLAS & CO – GRACELAND, G-TEAM

Aim of the study

The detection and quantitative analysis of the major bioactive ingredients of the olive oil as well as the determination of the total amount of hydroxytyrosol and its derivatives in order to investigate whether this olive oil sample meets the criteria of the European Food Safety Authority (EFSA) for olive oil polyphenols (health claim (EC) 432/2012).

A. EXTRACTION / SAMPLE PREPARATION

For the isolation of biophenols from the olive oil samples **OLE1375**, liquid-liquid extraction method was followed with the use of centrifuge. The polyphenolic extract evaporated to dryness under vacuum at 40 °C, weighted and then analysis was followed.

Additionally, in order to ascertain if the analyzed olive oil samples can bear the health claim approved by EFSA (**EU regulation 432/2012**) (*see below*), the biophenols extracts obtained from the extraction procedure described previously were subjected to acid hydrolysis [Mastralexi et al., 2014. Addressing analytical requirements to support health claims on "olive oil polyphenols" (EC Regulation 432/2012), *J Agric Food Chem*, 26;62(12):2459-61] and forwarder for HPLC analysis.

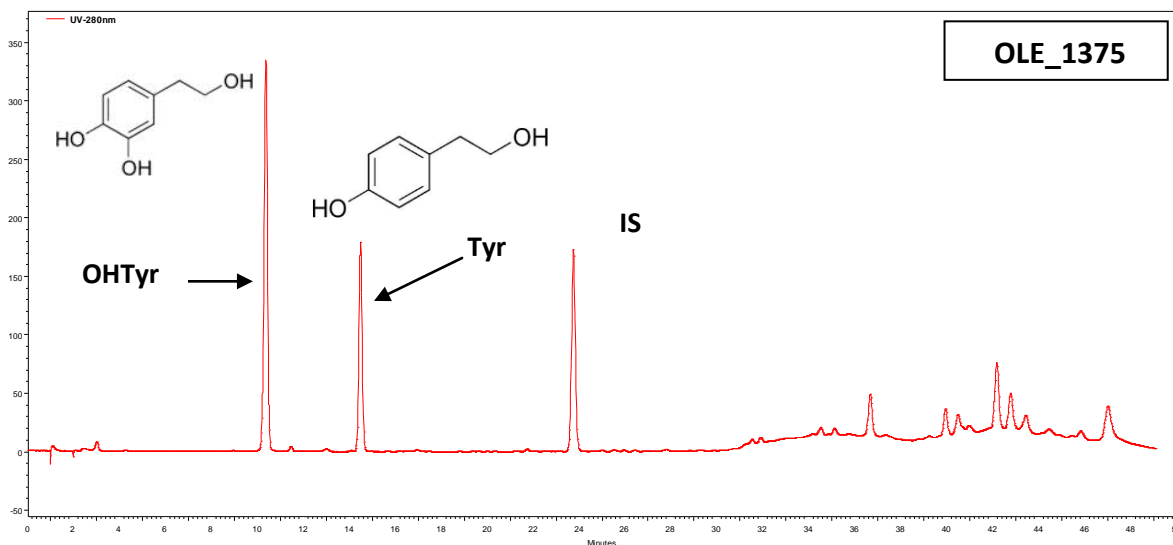
B. HPLC-DAD ANALYSIS

The HPLC-DAD technique was used for the analysis of **OLE1375** aiming to a) the qualitative profiling of the extracts under different wavelengths, and b) the quantitation and relative quantitation of representative biophenols.

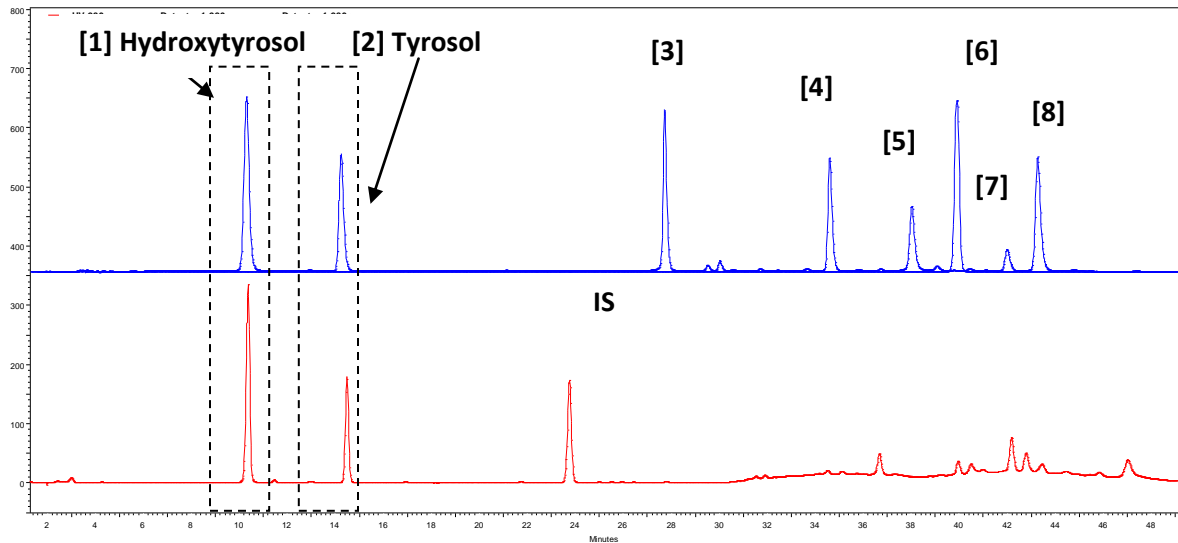
Two different methods were used for the determination of Hydroxytyrosol (**OHTyr**), Tyrosol (**Tyr**) and derivatives, and for secoiridoids, flavonoids and other polyphenols (*see experimental details*).

Results

B1) Detection of Hydroxytyrosol and Tyrosol

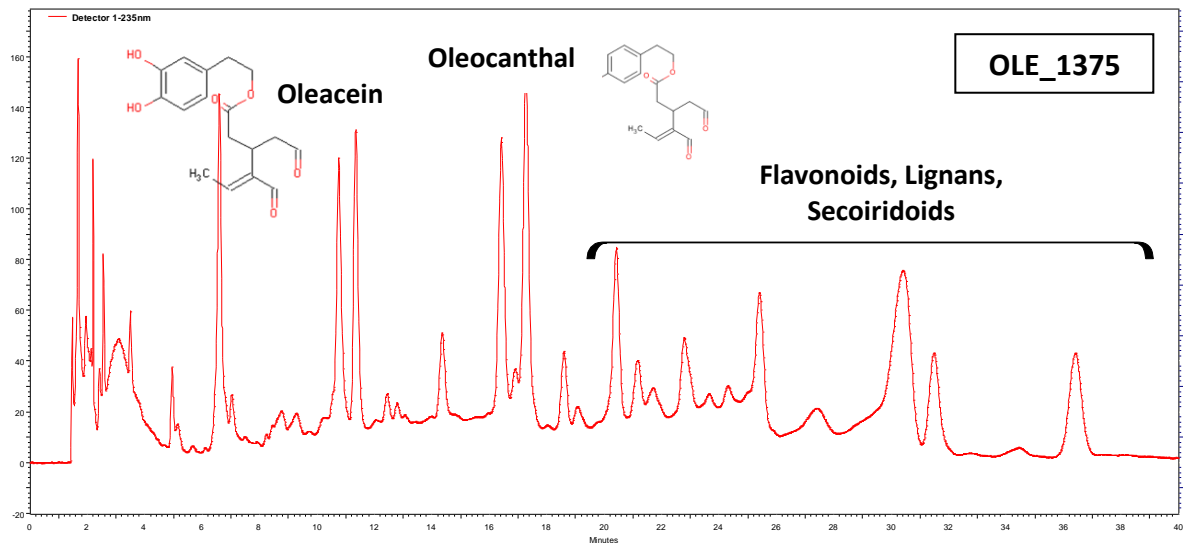


Picture 1: Chromatogram RP-HPLC-DAD (280nm) of the analysed sample **OLE_1375**, where the peaks pointed are the ones that correspond to Hydroxytyrosol and Tyrosol respectively.

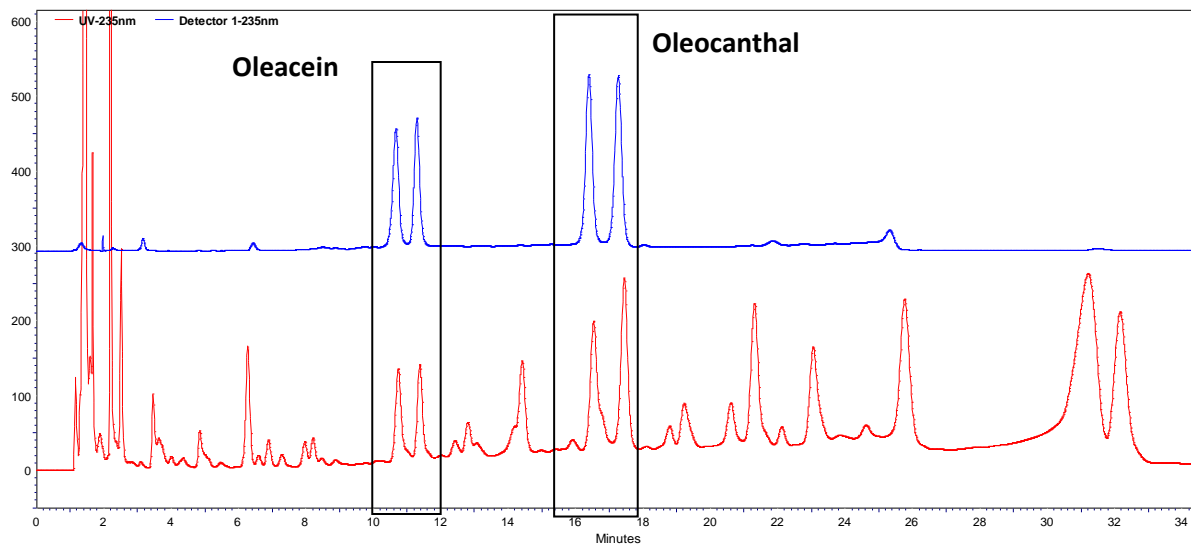


Picture 2: Chromatogram RP-HPLC-DAD at 280nm (red line) of the analysed sample, **OLE_1375**. The blue line corresponds to the chromatogram of a reference compounds solution at 280 nm (**1:** Hydroxytyrosol, **2:** Tyrosol, **3:** Verbascoside, **4:** Oleuropein, **5:** Luteolin, **6:** Pinoresinol, **7:** Acetoxypinoresinol, **8:** Apigenin).

B2) Detection of Oleacein and Oleocanthal



Picture 3: Chromatogram RP-HPLC-DAD (235 nm) of the analysed sample **OLE_1375**, where the peaks mentioned correspond to Oleacein and Oleocanthal. The region of elution of other important bioactive molecules is also mentioned, such as flavonoids, lignans and secoiridoids.



Picture 4: Chromatogram of the analysed sample **OLE_1375** at 235 nm (red line). The blue line corresponds to the chromatogram of the solution of the reference compounds Oleacein and Oleocanthal.

Table 1: Quantitative and semi-quantitative HPLC analysis of the four olive oil extracts.

Sample Code	HT (mg/Kg)	T (mg/Kg)	OLEA (mg/Kg)	OLEO (mg/Kg)
OLE_1375 (n=3)	40.12	32.71	52	107

Table 2: Quantitative HPLC analysis of the hydrolyzed olive oil extracts.

Sample Code	mg HT and T/20 g Olive oil	mg HT, T and derivatives/20 g Olive ^[1]
OLE_1375 (n=3)	532	1262

[1] Mastralexi et al., 2014. Addressing analytical requirements to support health claims on "olive oil polyphenols" (EC Regulation 432/2012), *J Agric Food Chem*, 26;62(12):2459-61.

MAJOR OBSERVATIONS AND COMMENTS

- As shown in the chromatogram at 280 nm (**Picture 1**), which is an indicative wavelength for the detection of phenyl alcohols, the highest levels of Hydroxytyrosol and Tyrosol, in total, are observed in **TPF-OLE_1375**. More specifically, based on the absolute quantification of the two phenyl alcohols, **OLE_1375** was found to contain **40.12 mg Hydroxytyrosol/Kg olive oil** and **32.71 mg Tyrosol/Kg olive oil**.

It is worth mention that Hydroxytyrosol and Tyrosol are considered from EFSA the functional chemical units of olive oil biophenols responsible for protective effects against LDL oxidation. In specific, EFSA recommends that 5 mg of Hydroxytyrosol and its derivatives (e.g. Oleuropein complex and Tyrosol) in olive oil should be consumed daily. Olive oils with a concentration of these biophenols over 5 mg / 20 g of olive oil can bear the health claim approved by EFSA.

- Regarding the 235 nm (**Picture 3 and 4**), based on the relative quantification of the two secoiridoids, was found to have Oleacein and Oleocanthal approximately **52** and **107 mg/Kg olive oil**, respectively.

Oleacein and Oleocanthal are secoiridoid derivatives of Hydroxytyrosol and Tyrosol respectively, which occur in high levels in the polyphenol fraction of extra virgin olive oil. They can provide the Hydroxytyrosol/Tyrosol units and recent studies associate these compounds with strong anti-inflammatory activity.

- Based on the results of acid hydrolysis^[1] (**Table 4**), **OLE_1375** was found to contain, on the date of analysis, **1262 mg Hydroxytyrosol and derivatives/Kg olive oil**. Consequently, the analysed olive oil sample **OLE_1375** meet the specifications of the EU regulation 432/2012 and can bear the health claim approved by EFSA (<http://www.gcsf.gr/media/trofima/reg-432-2012.pdf>), since it contains > 5 mg **Hydroxytyrosol and derivatives/Kg olive oil**.
- Moreover, **TPF (total polyphenolic fragment)** extract of the tested sample are characterised by the presence of other bioactive compounds, such as flavonoids

(e.g. luteolin, apigenin), lignans (e.g. pinoresinol, acetoxypinoresinol) and other secoiridoids (**Picture 2 and 4**).