# Chromatographic characterization of polyphenolic profiles of wastes from olive oil and winemaking industries


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## Introduction

Nowadays, the scientific community is increasingly concerned in the development of new strategies to recover and reuse essential compounds present in agro-food wastes. Among other matrices, olive oil and winemaking wastes have stirred up great interest as a source of products of high added value for chemical, pharmaceutical, or nutritional applications. In this regard, polyphenols are especially relevant because of their health promoting properties, such as strong antioxidant, antibiotic, anti-inflammatory and antineoplastic activities.

This work is focused on the extraction and characterization of the polyphenolic content of residues from olive oil and wine industries as a first step of waste revalorization. The recovery of target compounds by liquid extraction has been investigated using several techniques including stirring, ultrasound-assisted, microwave-assisted and pressurized liquid extraction. Experimental design has been applied to gain information on the extractive behavior of compounds and matrices from a reduced set of experiments. Results have shown significant differences depending on the nature of analytes and matrices.

The resulting extracts have been analyzed by liquid chromatography with UV spectroscopy and mass spectrometry detection. The separation have been carried out by reversed-phase mode in a C18 column, using an elution gradient based on 0.1% (v/v) formic acid aqueous solution and acetonitrile as the components of the mobile phase. UV detection at 280 nm has shown the great complexity of extracts. Further identification of relevant components by LC-MS has revealed that luteolin and *p*-coumaric acid are abundant in olive oil samples while caffeic acid and hesperidin are present in winemaking by-products.

## Objectives

1. **Optimization of the extraction procedure for the recovery of polyphenols in the waste samples**
   - Techniques:
     - Ultrasound-assisted extraction (UAE)
     - Microwave-assisted extraction (MAE)
     - Pressurized liquid extraction (PLE)
   - Approach based on experimental design and statistic analysis for assessing the significance of factors

2. **Optimization of the liquid chromatographic separation of waste extracts**
   - Preliminary study of the separation by HPLC-UV
   - Approach based on experimental design with multicriteria decision making

3. **Characterization of the waste extracts**
   - Estimation of contents of polyphenolic families by HPLC-UV
   - Identification of relevant compounds by UHPLC-MS.

## Experimental section

### Olive oil and winemaking by-products

| *Olive oil* | *Type of residue* | *Origin* |
|---|---|---|
| O1 | *Alperujo* | Borges |
| O2 | *Alperujo* | Capricho Andaluz |
| O3 | *Alperujo* | Albelda |
| O4 | *Orujo* | Faiges Navamorales |
| O5 | *Orujo* | Borges |
| O6 | *Fatty acids* | Borges |
| ***Wine*** | ***Type of residue*** | ***Origin*** |
| L1 | Lees | Bodegas Torres (red wine) |
| L2 | Lees | Bodegas Torres (white wine) |
| L3 | Lees | Felix Solis |
| L4 | Lees | Felix Solis |

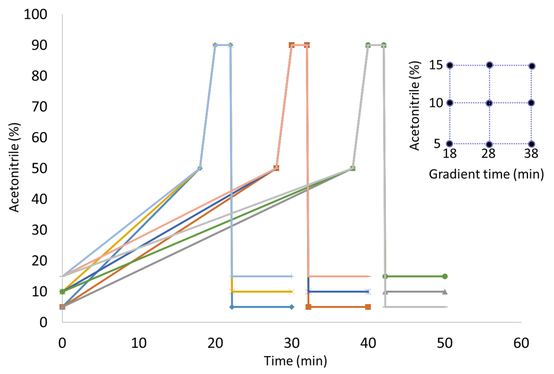
*Example of an olive oil alperujo sample*

### Experimental design

*Factors for optimizing the extraction*
- Organic solvent percentage
- HCl percentage
- Extraction time
- Temperature

*Factors for optimizing the separation*
- Organic solvent percentage
- Gradient time

**Statistics evaluation of the significance of factors and interactions**
- Analysis of variance




*Example of a 2-factor at 3-level design for the optimization of the separation gradient*

### HPLC-UV

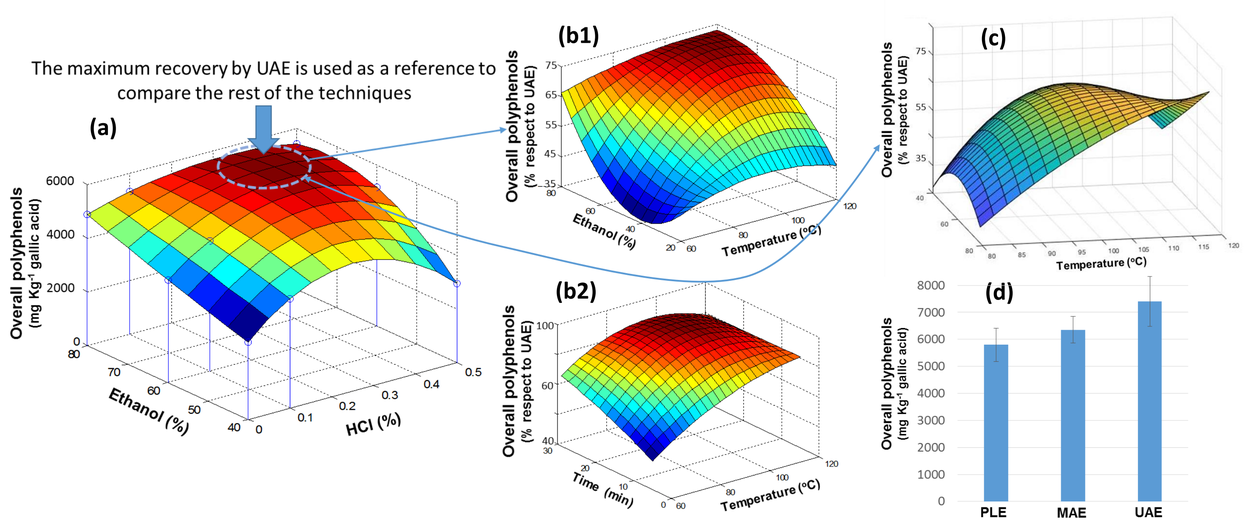
- HPLC Agilent Series 1100 HPLC chromatograph with diode array detection
- Separation column Kinetex C18 (100 x 4.6 mm I.D., 2.6 µm particle size).
- Mobile phase: 0.1% aqueous formic acid solution and acetonitrile
- Flow rate: 0.4 mL $min^{-1}$
- Detection wavelength: 280 nm (phenolic acids and flavanols); 310 nm (hydroxybenzoic acids and stilbenes); 370 nm (flavonoids)

### HPLC-MS

- Accela UHPLC with Q-exactive Orbitrap detection (Thermo Fisher Scientific)
- Separation column Ascentis Express C18 (150 x 2.1 mm, 2.7 µm)
- Mobile phase: 0.1% aqueous formic acid solution and acetonitrile
- Flow rate: 0.8 mL $min^{-1}$
- Ionization mode: negative
- Full scan MS: m/z 100-1,500. Resolution: 70,000 FWHM at m/z 200

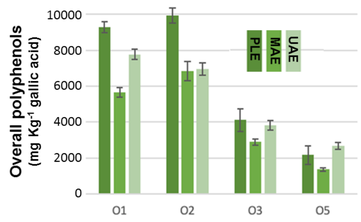
## Results and discussion

### Optimization of the extraction by UAE, MAE and PLE

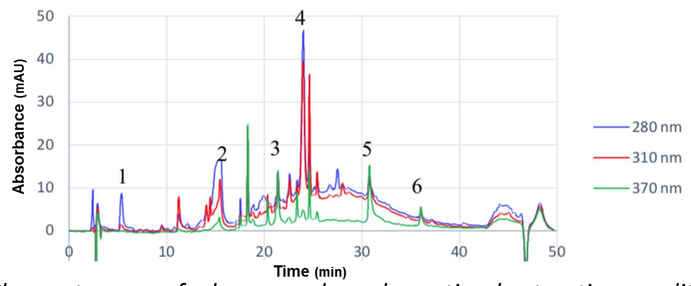



*Experimental design to study the performance of the extraction techniques for the recovery of the polyphenols from lees samples. Influence of some experimental variables (a) UAE, (b1) and (b2) MAE (c) PLE and (d) Comparison of techniques under optimal conditions.*

A similar study has been carried out for the olive oil residues. In this case, the behavior is different and PLE seems to be a more appropriate technique.




### Study of compositional profiles of polyphenols by HPLC-UV and LC-MS

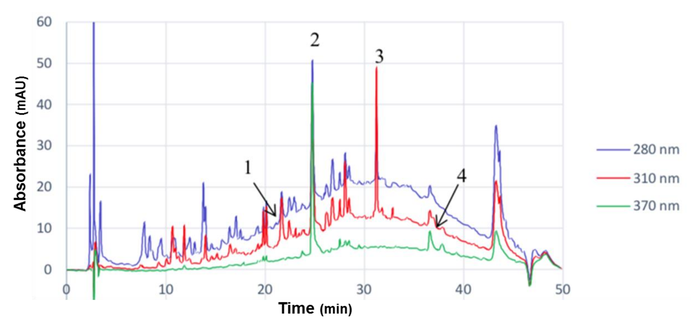



*Chromatogram of a lees sample under optimal extraction conditions.*

*Peak assignment: 1 = gallic acid; 2 = caffeic acid; 3 = rutin; 4 = hesperidin; 5 = luteolin; 6 = kaempferol*

*Major ions of lees samples pending to be identified*

| $t_R$ (min) | Formula | m/z |
|---|---|---|
| 5.25 | $C_{18}H_{23}O_2$ | 271.16 |
| 6.39 | $C_{15}H_{17}O_8$ | 325.09 |
| 10.1 | $C_{24}H_{49}O_{10}$ | 497.33 |
| 11.7 | $C_{23}H_{23}O_{13}$ | 507.11 |
| 11.8 | $C_{48}H_{67}O_5$ | 723.05 |
| 12.6 | $C_{48}H_{67}O_5$ | 723.5 |




*Chromatogram of an oil sample under optimal extraction conditions.*

*Peak assignment: 1 = p-coumaric acid; 2 = luteolin; 3 = resveratrol 4 = kaempferol*

*Major ions of oil samples pending to be identified*

| $t_R$ (min) | Formula | m/z |
|---|---|---|
| 4.28 | $C_{17}H_{27}O_{11}$ | 407.15 |
| 4.61 | $C_9H_{13}O_4$ | 185.08 |
| 4.77 | $C_9H_{11}O_5$ | 199.06 |
| 5.83 | $C_{16}H_{21}O_{11}$ | 389.10 |
| 7.02 | $C_{17}H_{23}O_{11}$ | 403.12 |
| 7.45 | $C_{16}H_{25}O_{10}$ | 377.14 |
| 8.2 | $C_{13}H_{14}O$ | 186.11 |
| 12.2 | $C_{20}H_{29}O_{13}$ | 477.16 |
| 12.63 | $C_{48}H_{67}O_5$ | 723.5 |
| 13.41 | $C_{15}H_{18}O$ | 214.14 |
| 15.87 | $C_{16}H_{20}O$ | 228.16 |
| 18.59 | $C_{18}H_{35}O_5$ | 331.25 |

## Conclusions

✓Optimal extraction conditions for the recovery of polyphenols from lees correspond to UAE using ethanol/HCl/water 60/0.1/39.9 (*v/v/v*) as the solvent.

✓Optimal extraction conditions for the recovery of polyphenols from olive oil residues correspond to PLE. However, for practical reasons, UAE is recommendable when dealing with industrial applications, also using ethanol/HCl/water 60/0.1/39.9 (*v/v/v*) as the solvent.

✓ Chromatographic profiles of both lees and olive oil wastes are rich in compounds such as gallic acid, rutin, hesperidin, luteolin, etc. thus resulting in excellent sources to isolate and purify these polyphenols.

✓Further studies by LC-MS will be needed to identify the chemical components responsible for some relevant unknown peaks.

## References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