

Exhausted olive pomace phenolic-rich extracts obtention, a first step for a biorefinery scheme proposal



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CONTENT



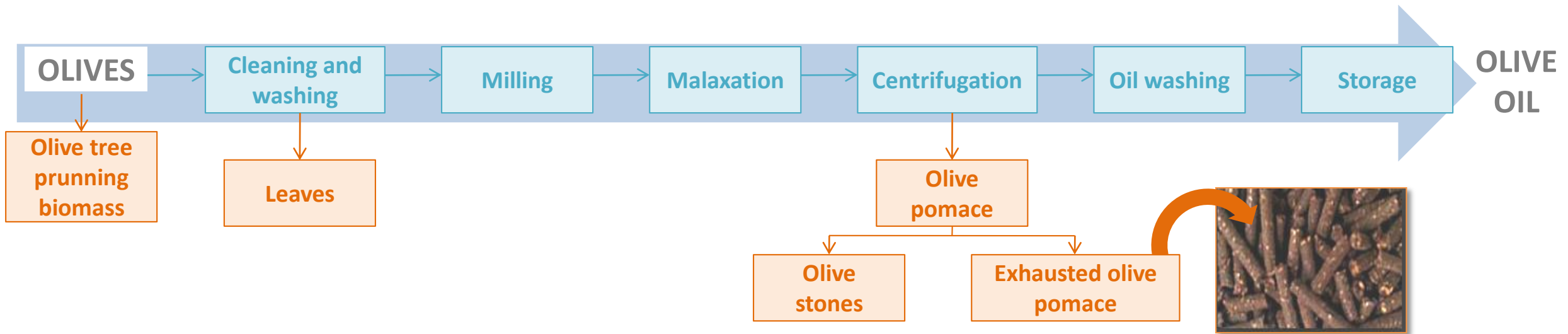
- ★ Introduction
- ★ Objective
- ★ Material and Methods
 - Raw material
 - Experimental conditions
- ★ Results
- ★ Conclusions
- ★ References and acknowledgments



INTRODUCTION



- **Spain** is the world's leading producer and exporter of olive oil 
- 2.5 million ha of olives are cultivated in Spain 
- Olive oil production generates every year **4.2 million tons/year of residues** [1] (see scheme) and there by it is a big deal for the Spanish industry.

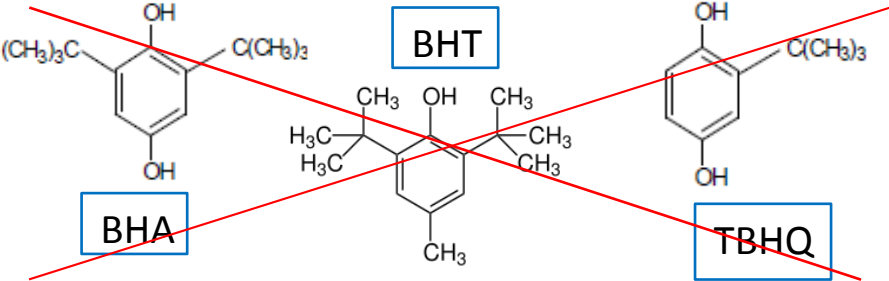


Scheme production of olive oil and waste generated (orange squares)



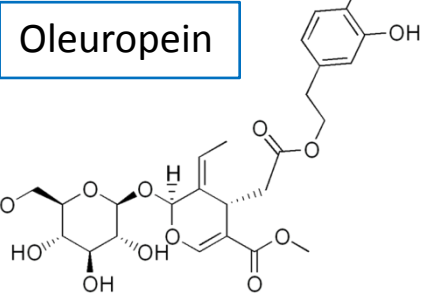
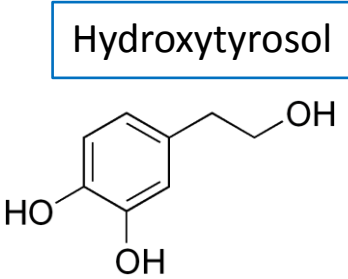
INTRODUCTION

Nowadays, an increasing demand on natural antioxidants exists by both consumers and industry. These compounds can replace synthetic antioxidants that have some side effects.



SYNTHETIC ANTIOXIDANTS

REPLACING



NATURAL ANTIOXIDANTS

Advantages of NATURAL ANTIOXIDANTS from olive biomass

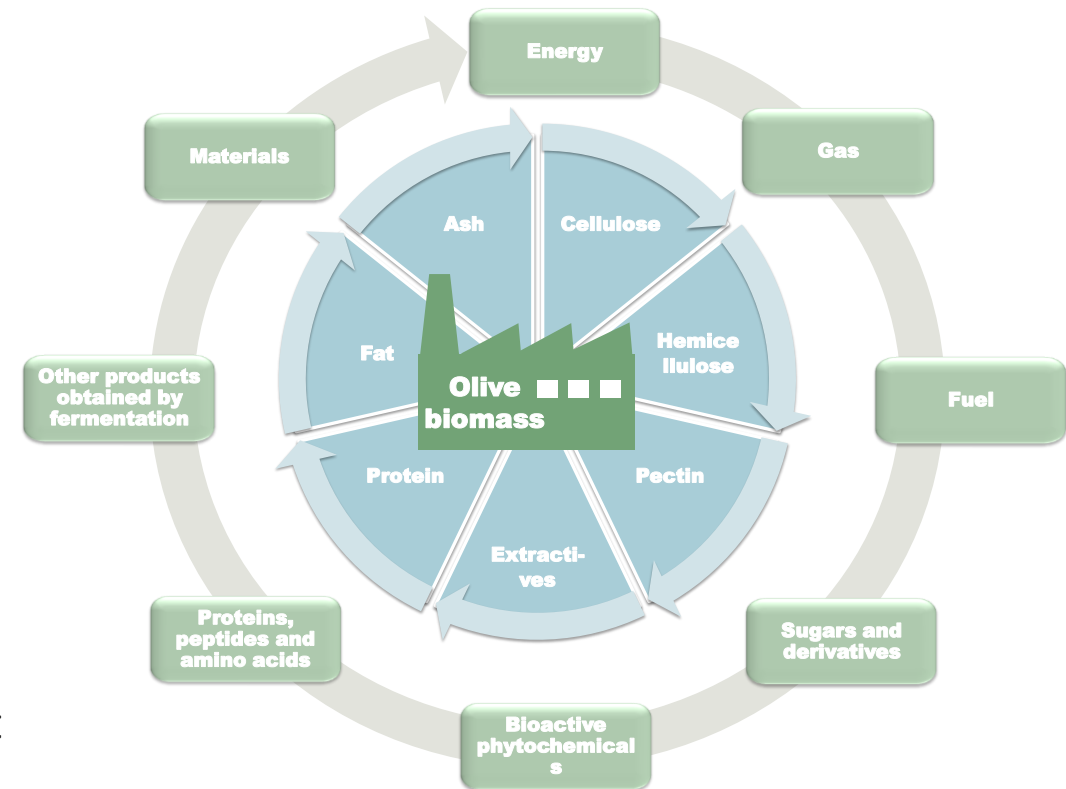
- Potential health benefits.
- Many applications for the pharmaceutical and food industries.
- Hydroxytyrosol is one of the antioxidants of olive oil that confers protection of blood lipids from oxidative stress [2].
- The European Food Safety Authority has included as novel food ingredient synthetic hydroxytyrosol because it is safe under the proposed uses and use levels to fish, vegetable oils and margarines [3].



INTRODUCTION



- Nowadays, the development of a biorefinery that contributes to the integral use of the resources of the olive grove is valued as of great social, environmental, and economic interest.
- Among these sources, **exhausted olive pomace** (EOP) is considered a promising feedstock within the context of biorefinery for the production of bioenergy and valuable chemicals, including natural antioxidants.
- The latter use can provide with an extra income to the current application of EOP as a low-cost fuel for domestic and industrial heating.



Potential products obtained in a biorefinery based on olive biomass



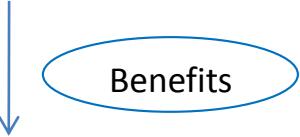
OBJECTIVE

The aim of this work was to obtain antioxidants from EOP. The extraction experiments to achieve this objective are:

1) Ultrasound-assisted extraction (UAE) was performed using sequential extraction with water and 70% acetone as solvents.

UAE is considered an efficient extraction technique

- Reduces extraction time
- Reduces solvent and energy consumption



- Increasing yields
- Savings on an industrial scale

2) Maceration using water at 85 °C under agitation.



MATERIAL AND METHODS



Raw material and chemical composition

- Olive pomace industry “Spuny SA” (Castellar, Jaén)
- Pitted and pelletized



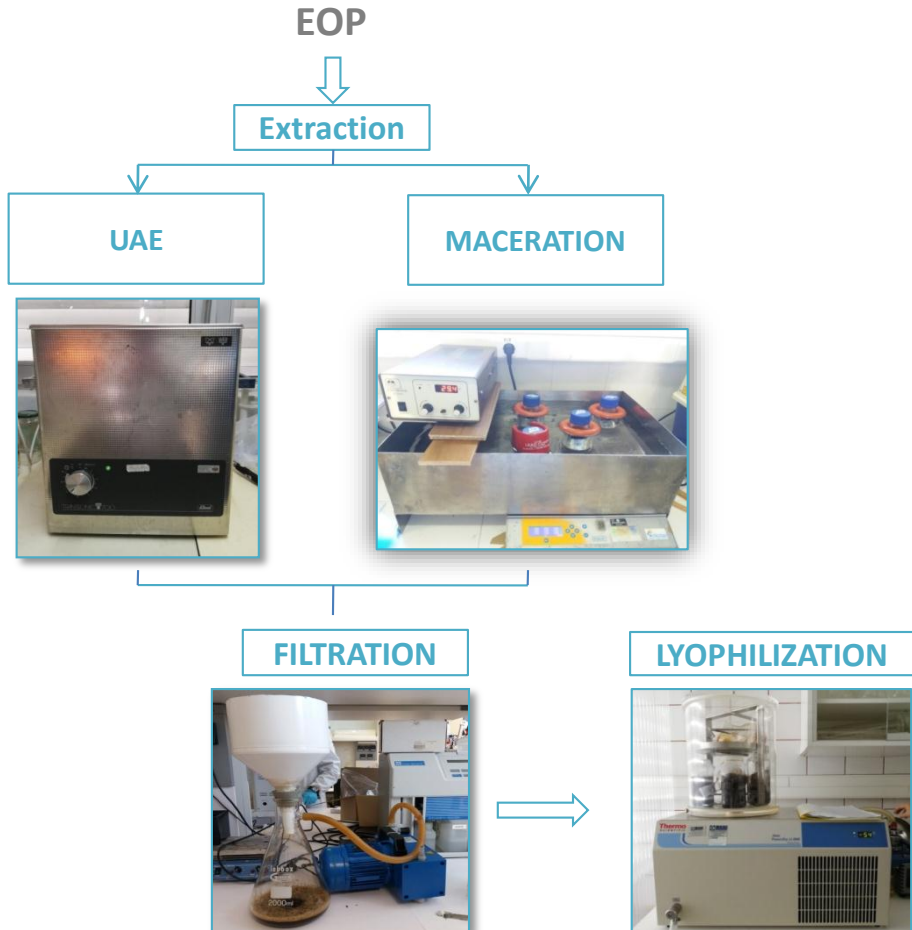
- Extractives
- Acid Insoluble Lignin (AIL)
- Acid Soluble Lignin (ASL)
- Carbohydrates
- Acetyl groups
- Ash

Experimental conditions

Extraction method	Solvent	Time (min)	Temperature (°C)	Solids (%)
UAE	Water	30	30	10
UAE	70% acetone	30	30	10
Maceration	Water	90	85	10



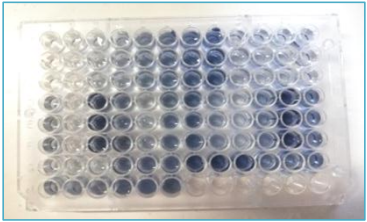
METHODS



ANALYTICAL DETERMINATION

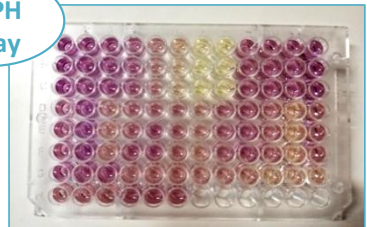
Lyophilized extracts

TOTAL PHENOLIC ONTENT

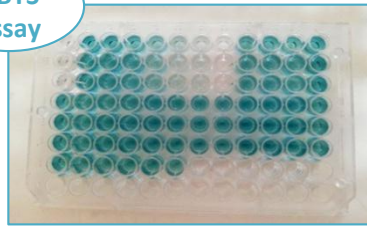


ANTIOXIDANT ACTIVITY

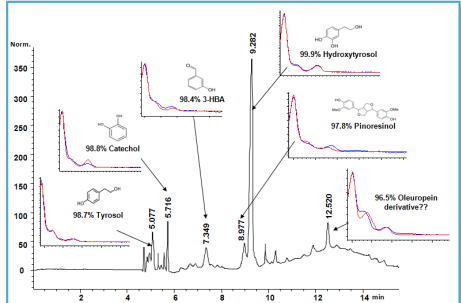
DPPH assay



ABTS assay



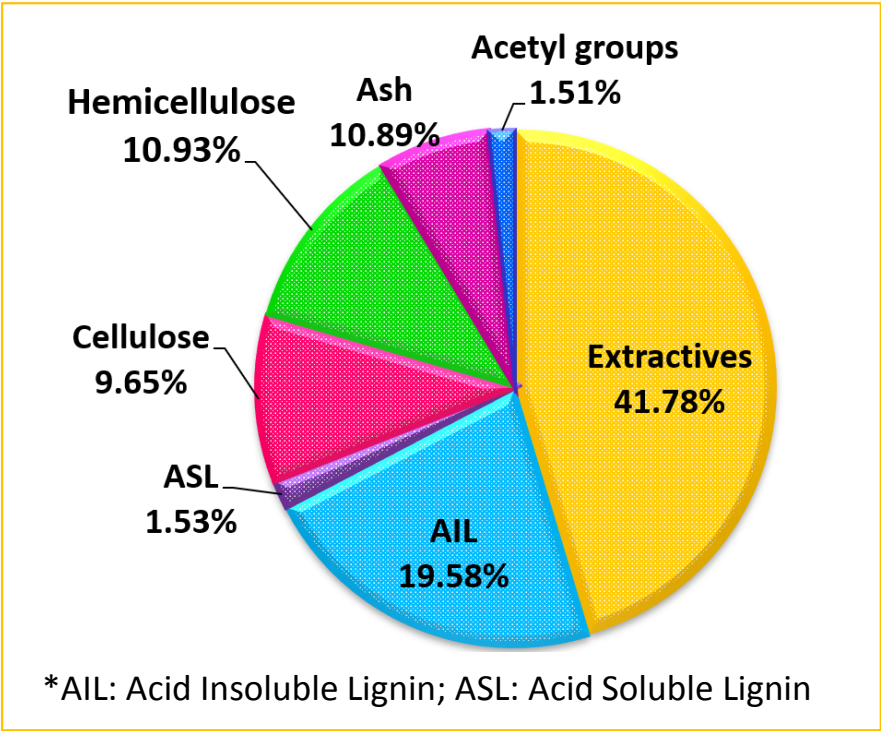
CAPILLARY ELECTROPHORESIS PROFILES





RESULTS

EOP chemical composition



- The chemical composition of EOP was: 41.8% of extractives, 20.6% of carbohydrates and 21.8% of lignin.
- Since the major component are **extractives**, its valorization is crucial.
- Thereby, the antioxidant fraction of the extractives was obtained by different methods as a first step for the valorization of EOP.



RESULTS

Total phenolic content and antioxidant activity of the liquid extracts

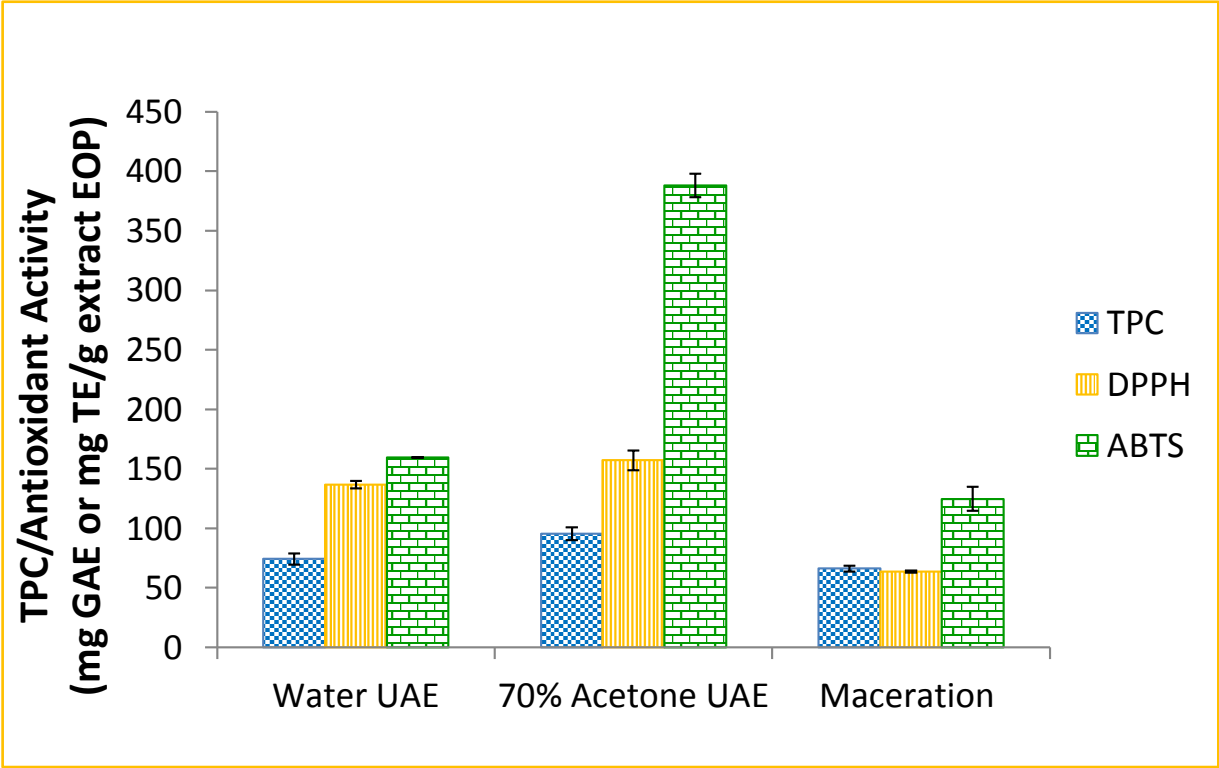
Extraction method	Solvent	Sample	TPC g GAE/L	DPPH g TE/L	ABTS g TE/L
UAE	Water	Extract 1	1.37 ± 0.01	1.38 ± 0.09	3.43 ± 0.05
		Extract 2	1.01 ± 0.06	0.98 ± 0.05	1.50 ± 0.12
		Extract 3	0.50 ± 0.03	0.50 ± 0.01	0.99 ± 0.05
		Mixture	0.96 ± 0.03	0.98 ± 0.01	1.94 ± 0.01
UAE	70% acetone	Extract 1	1.28 ± 0.03	1.54 ± 0.01	3.43 ± 0.04
		Extract 2	0.83 ± 0.09	0.90 ± 0.03	1.91 ± 0.05
		Extract 3	0.77 ± 0.00	0.55 ± 0.02	0.83 ± 0.11
		Mixture	0.97 ± 0.01	1.00 ± 0.07	2.04 ± 0.03
Maceration	Water	Extract	4.22 ± 0.1	3.04 ± 0.27	6.77 ± 0.47

- Using 10% solids load and UAE at 30 °C, it is recommended to perform three sequential extractions in order to recover the highest possible concentration of phenolic compounds.
- Using water extraction through maceration at 80 °C, higher amounts of phenolic compounds were solubilized in the liquid fraction in a single step; i.e., 4.22 GAE vs. 2.88 GAE using UAE with both solvents if the extracts were dried.



RESULTS

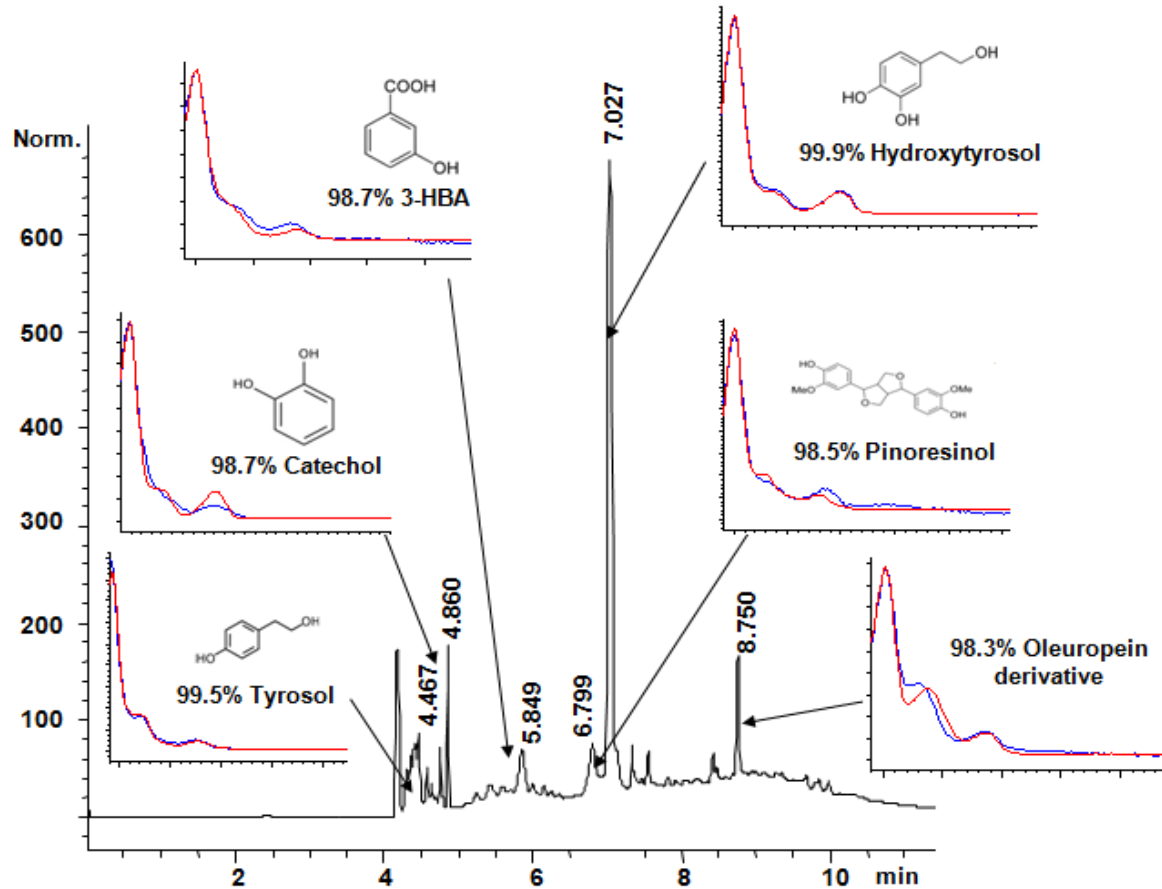
Total phenolic content and antioxidant activity of the liquid extracts



- When the results were expressed in terms of extract, UAE with water-acetone revealed to be more effective than with water; 95.7 versus 74.4 mg GAE/g extract, respectively.
- This can be explained due to the amounts of solids was higher using the latter methods, reducing the purity of the extract.
- The extract obtained in an agitated water bath at 85 °C showed a TPC of 65.9 mg GAE/g extract.
- The antioxidant activity of the extracts revealed to be in accordance with the TPC, being the results higher for UAE extracts, particularly, using water-acetone.

RESULTS

Phenolic profile of the water-acetone extract

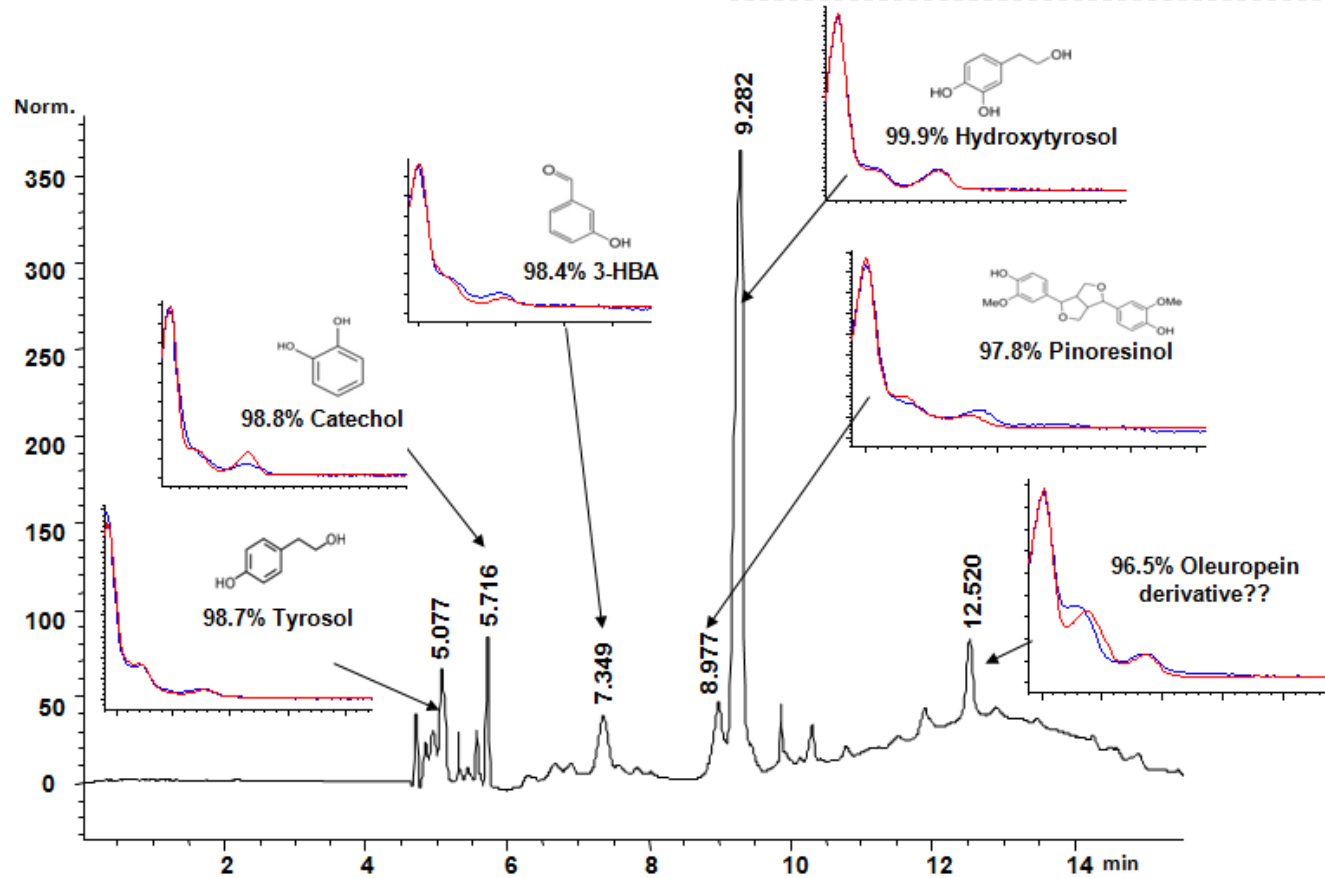


- The electrophoretic analysis revealed the presence of hydroxytyrosol as major compound of the extract.
- This agreed with other studies that found hydroxytyrosol in olive pomace (OP).
- Remarkably, our results suggest that this compound is highly resistant since OP is subjected to drying and solvent extraction to recover the olive pomace, obtaining EOP as final waste.

Electropherograms at 200 nm of the water-acetone extract obtained by UAE

RESULTS

Phenolic profile of the water extract



Electropherograms at 200 nm of the water extract obtained by maceration with constant stirring

- The electrophoretic profile was similar to the aqueous extract and the major compound of the extract was again hydroxytyrosol.
- This can be explained due to its high polarity being extracted by water and its mixture with acetone.



CONCLUSIONS

Results showed that EOP presents a significant amount of phenolic compounds, independently of the extraction conditions.

While the highest solubilization of phenolic compounds was obtained using water extraction at 80 °C, UAE with 70% acetone revealed to be more effective for the obtention of an extract richer in antioxidants.

Hydroxytyrosol was the major compound found in all the extracts, which is also considered one of the most powerful antioxidants.

EOP is a promising source of added-value phenolic compounds with antioxidant activity, in particular hydroxytyrosol, and its obtention is worth-merit before the exploration of this waste for bioenergy production.



REFERENCES AND ACKNOWLEDGMENTS

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- European Commission Commission Regulation (EU) No 1018/2013 of 23 October 2013 amending Regulation (EU) No 432/2012 establishing a list of permitted health claims made on foods other than those referring to the reduction of disease risk and to children's development and health. *Off. J. Eur. Union L 282*. **2013**, 56, 43–45, doi:http://eur-lex.europa.eu/pri/en/oj/dat/2003/l_285/l_28520031101en00330037.pdf.
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