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Valorization of olive industry by-products for tracing and extraction of hydroxytyrosol using various green extraction technologies

Xynos N., Argyropoulou A., Aligiannis N., Polychronopoulos P., Abatis D., Skaltsounis A.L.

Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis Zografou, 15771, Athens, Greece

Introduction

Currently, there is keen interest in dietary polyphenols due to their antioxidant capacity and consequent benefits to human health. At least 36 structurally distinct olive phenolics have been identified¹. Studies have demonstrated that olive phenolic compounds have positive effects on certain physiological parameters, such as plasma lipoproteins, oxidative damage, inflammatory markers, platelet and cellular function, antimicrobial activity and bone health. Moreover, according to European Food Safety Authority, certain claimed effects are approved regarding olive oils containing at least 5 mg of HT and its derivatives (e.g. oleuropein complex and tyrosol) in a moderate daily dose².

The purpose of this work was to exploit by-products of the olive industry for the production of an extract with high percentage in olive polyphenols, focusing mostly on its hydroxytyrosol (HT) content. Three by-products were obtained, table olive processing wastewater (TOPW), olive paste (OP) and rinse water (RW) from two specific processes: table olive natural debittering process and olive oil two-phase cold extraction. The aim was to assess these sources for their polyphenolic content, as well as to compare various green extraction and/or fractionation technologies. The final goal was to design an efficient extraction/fractionation procedure, leading to a final product rich in HT, destined for the market of nutraceuticals and functional foods.

Plant material and extraction apparatuses

TOPW occurs during the natural debittering process of table olives and it is normally disposed at the end of the process which lasts several months. RW is the water that is used for the final rinsing of the olive oil production process, destined to refine the latter. OP is the residual de-oiled olive paste occurring in two-phase olive oil production units. TOPW (Amfissis variety, *Olea europaea* var. rotunda), OP (Kalamon variety, *Olea europaea* var. sativa), and RW (Kalamon variety, *Olea europaea* var. sativa), were collected in 2011 from the regions of Lakonia and Evia. OP was submitted to various extraction procedures, including maceration (MAC), ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE). The latter was performed on a Separex 1-2 lt. pilot apparatus. Moreover, Dionex ASE 300 apparatus was used for the pressurized liquid extraction (PLE) and the subcritical water extraction (SWE) of OP. Adsorptive resin technology (ART) was implemented for the recovery of the polyphenols regarding TOPW and RW, using XAD-4 macroporous resins in packed column provided by Rohm and Haas. All extracts were submitted to thin layer chromatography (TLC). The quantitative determination of HT in the extracts and fractions was performed with a Thermo Finnigan HPLC system (Thermo Finnigan, San Jose, CA) coupled with a Spectra System UV6000LP PDA detector. The structure determination was also based on MS. High Resolution. Mass spectra were recorded on a LTQ-Orbitrap Discovery (ThermoFinnigan).

Extraction protocols and results

The three by-products were evaluated for their content in hydroxytyrosol using HPLC. Several extraction methods and protocols were applied in the case of OP (solid), while liquid RW and TOPW were fed to macroporous resin columns in order to recover the phenolic content. An exhaustive extraction scheme was performed for each raw material in order to evaluate its HT content. Total HT recovery was verified using TLC and HPLC. Thus, OP was found to contain 0.08% HT, RW contained 0.002% HT while TOPW contained 0.1% HT. All experiments and results are illustrated in Table 1.

Table 1. Extraction methodologies for the various by-products and results

RAW MATERIAL	EXTRACTION METHOD	SOLVENT/PROTOCOL	DRY YIELD %	%HT IN EXTRACT	EXTRACT ASPECT
OLIVE PASTE (60% HUMIDITY)	SFE-RATIO 0-200	SC-CO ₂	0.92	0.1	OILY SEMISOLID
OLIVE PASTE (60% HUMIDITY)	SFE RATIO 0-150	SC-CO ₂ +3% EtOH	1.23	0.32	OILY SEMISOLID
OLIVE PASTE (20% HUMIDITY)	LYOPHILIZATION/ SFE RATIO 0-150	SC-CO ₂ +3% EtOH	12.19	0.05	OILY LIQUID
OLIVE PASTE (60% HUMIDITY)	BOIL/MACERATION	H ₂ O	4.00	0.06	PASTE
OLIVE PASTE (60% HUMIDITY)	MACERATION	EtOH 96°	7.06	0.68	PASTE
OLIVE PASTE (60% HUMIDITY)	PRESSURIZED LIQUID EXTRACTION	EtOH 96°,70°C	3.20	0.21	PASTE
OLIVE PASTE (60% HUMIDITY)	PRESSURIZED LIQUID EXTRACTION	SUBCRITICAL H ₂ O 190°C	8.65	0.30	PASTE
OLIVE PASTE (60% HUMIDITY)	ULTRASOUND/MICROWAVE	MeOH	10.83	0.71	PASTE
OLIVE PASTE (60% HUMIDITY)	MACERATION IN pH=1	H ₂ O/EtOH 50:50	6.99	0.69	SEMISOLID PASTE
RINSE WATER	XAD-4 RESIN PACKED COLUMN	EtOH 96°	0.03	1.97	PASTE
TABLE OLIVE PROCESS WATER	XAD-4 RESIN PACKED COLUMN	EtOH 96°	0.34	9.98	SEMISOLID PASTE

Acknowledgments

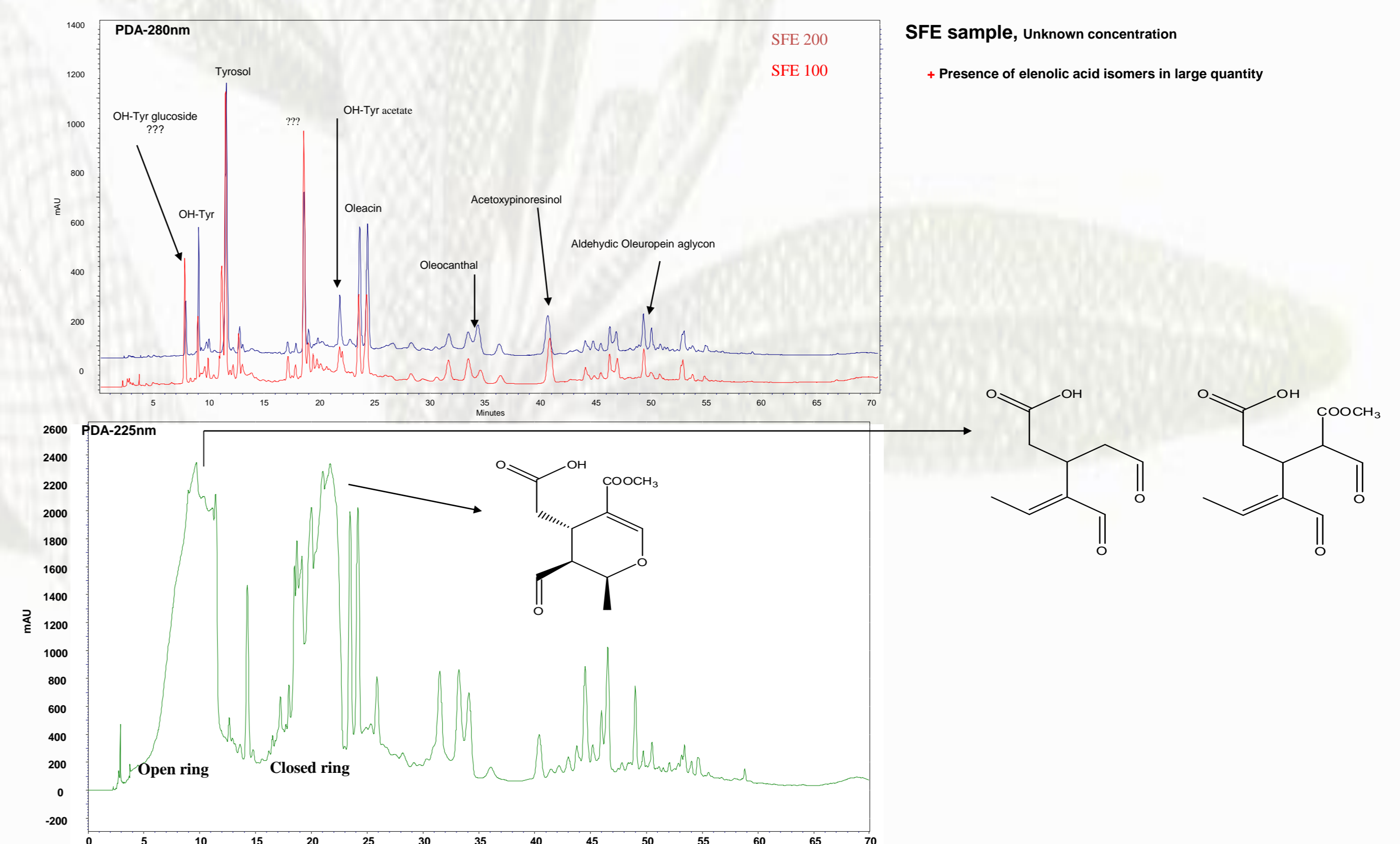
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References

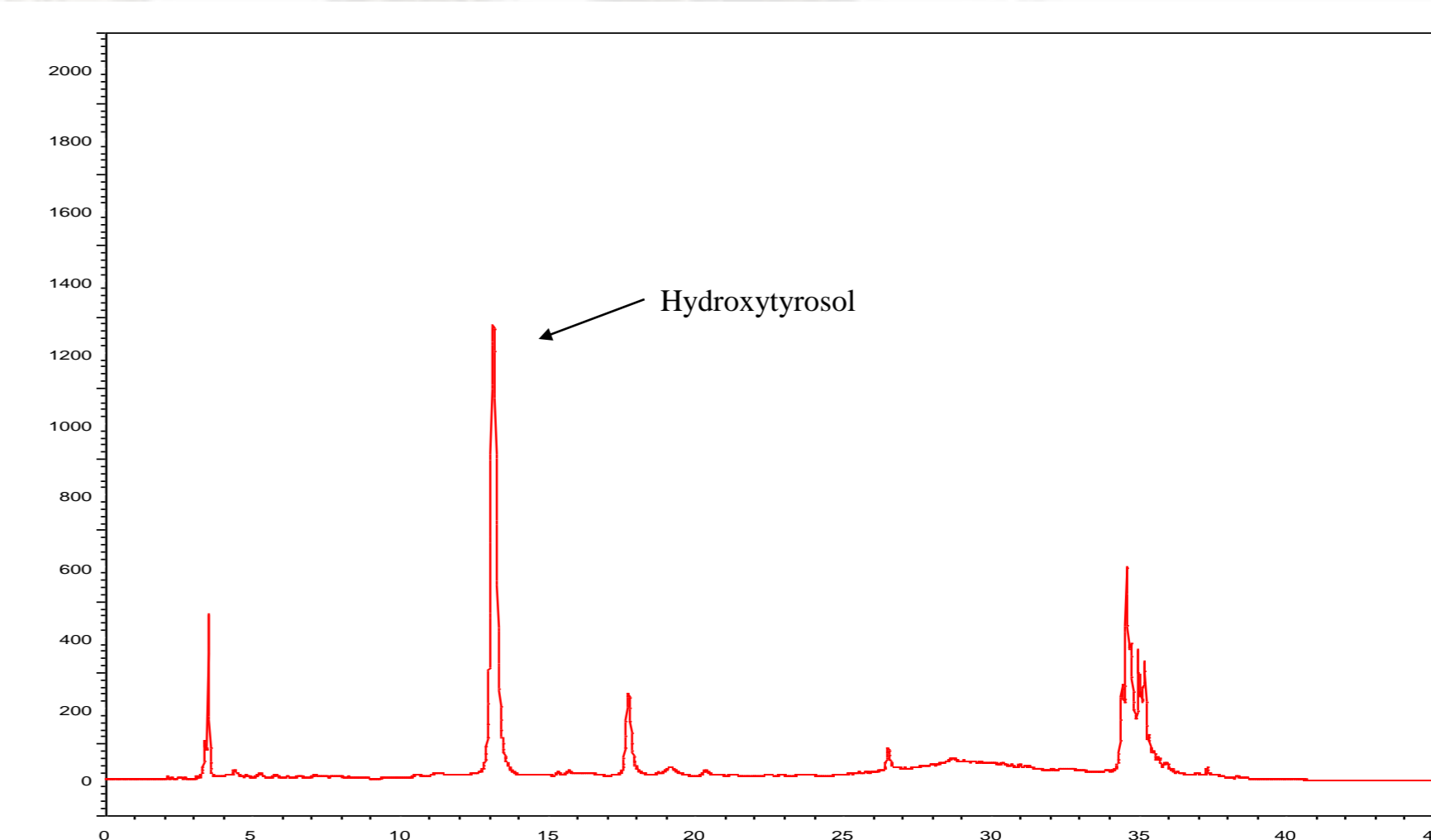
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Discussion

OP is a suitable material for supercritical CO₂ extraction, optimally with the addition of a small percentage of cellulose. SFE proved to be efficient to extract OH-Ty, though recovery was not high, especially when there was no EtOH as a co-solvent (1.53% vs 4.92%). This led to an extract containing 0.32% OH-Ty. The recovery was even better when lyophilization of the raw material preceded extraction (7.62%) and resulted to a lipophilic extract containing 0.05% OH-Ty. Yield was extremely enhanced due to the extraction of heavy lipophilic compounds. It was observed that, comparing to all the other methods, the extracts with SFE presented the most versatile profile in terms of polyphenols and some lipids. The LC-MS chromatograms of the SFE extracts that were obtained at 100 and 200 bar permitted to identify some of the compounds that are present, such as oleacein, oleocanthal, acetoxypinoresinol, aldehydic oleuropein aglycon, elenolic acid and its isomers etc :



LC-DAD chromatograms of the SFE extracts obtained at 100 and 200 bar



HPLC chromatogram of TOPW phenolic concentrate after ART treatment

Maceration in boiling water had a very poor recovery while maceration in EtOH presented the best recovery among all the methods used (60%) and resulted to an extract containing **0.68% HT**. Both procedures presented some filtration difficulties as the nature of the material was pasty. **PLE** with EtOH had a mediocre HT recovery though larger contact (3 cycles of 5 min. were performed) with the material would clearly increase significantly the recovery. The extract contained **0.21% OH-Ty**. This was even more evident concerning **SWE** at 190 °C were same (short) extraction scheme resulted a very good HT recovery. The extract contained **0.30% HT**. **UAE in combination with MAE** resulted in an extract containing **0.71% HT** and an optimal recovery. **SFE without the use of a co-solvent**, proved to be quite a non-polar extraction that provided an extract with only **0.1%** of HT, while **SFE with 3%EtOH** resulted in an extract with **0.32%** of HT. Lyophilization seemed to enhance the extraction of lipids, as humidity of OP fell from 60% to 20%. Concerning the use of **ART** for the liquid RW and TOPW, this was proved to be very efficient, as XAD-4 resin was able to retain the olive polyphenols (especially HT) very well, provided that a saturation study was performed so that maximum feed (for the polyphenols adsorption) and minimum EtOH quantity (for the desorption) are defined. The resins' capacity for the specific raw material has been determined and was 5 bed volumes (BV) for the adsorption step and 2 BV of EtOH for the desorption. An extract containing **9.98%** of OH-Ty was obtained from TOPW after concentrating polyphenols with the use of ART. The majority of the extracts had an aspect of a paste, slightly sticky. SFE extracts of OP differentiated being more lipophilic (it contained triterpenes and lipids). TOPW extracts were honey-like concerning the color and viscosity, but less sticky. In olfactive They present an odor of olives.

Conclusions

Three different olive oil industrial by-products have been evaluated for their content in HT. TOPW proved to be the more rich, followed by OP. RW was extremely poor in HT content. Several conventional or green extraction methods were implicated for the extraction of HT from OP, while for the liquid materials, TOPW and RW, the ART was chosen as the most appropriate in order to recover HT. Among all extraction methods for OP, the combination of UAE and MAE resulted the higher HT recovery, followed by simple maceration with EtOH, in contrast with aqueous extraction in boiling water. Acidic environment did not seem to improve the latter in terms of HT recovery. SWE in 190°C also resulted in a good recovery of HT, while SFE extractions did not yield significant amounts of HT. Regarding RW, its enrichment through the use of ART reached a factor of 1000 and yielded low amounts of an extract relatively richer in HT than OP extracts. Finally, the most rich by-product and effective method were combined in the case of TOPW enrichment in HT with the implementation of ART. By far the richest extract in HT was obtained in this case, while the whole procedure was relatively simple, requiring only a XAD-4 resin-packed column and resulting a rich concentrate of HT and olive polyphenols. This absolutely "green" extract, produced with the use of water, agricultural EtOH and food-grade macroporous resins, could be destined to the market of functional foods, nutraceuticals, cosmetics and phytotherapy.