EXTRACTION AND RECOVERY OF PHENOLIC COMPOUNDS FROM OLIVE LEAVES

Mª Yolanda Franco Pérez*, José Manuel Benito Moreno, Isabel Escudero Barbero

Área de Ingeniería Química, Departamento de Biotecnología y Ciencia de los Alimentos, Facultad de Ciencias, Universidad de Burgos. E-mail: mfp0048@alu.ubu.es

- **There is currently a great interest on the use of the residual biomass originated from the agricultural and food sectors as a bioresource instead as a waste, due to its high potential for the recovery of high added-value compounds.**
- **Olive leaves, an olive oil processing residue, contain high amounts of phenolic compounds with health-promoting properties, such as oleuropein, tyrosol and hydroxytyrosol, which can be extracted from olive leaves to be added later in processed foods and thus increase their healthy properties.**
- **In this work, the recovery of phenolic compounds was studied using solvent extraction of olive leaves, kindly provided by the cooperative "Pagos de Benaval" from olive trees of the variety "Serrana de Espadán", endemic of the Sierra de Espadán natural park (Castellón, Spain).**

MATERIALS AND METHODS

- **Preliminary experiments for the recovery of phenolic compounds present in previously ground and dried olive leaves (size less than 1 mm) were performed by solid-liquid extraction using several solvents (hexane, ethanol, methanol and ethyl acetate), in order to select the best one.**
- **The total phenolic content (TPC) in extracts was determined according to the Folin-Ciocalteau method. Phenolic profile in olive leaf extracts and quantitative determination of oleuropein (OT) and hydroxytyrosol (HT) were performed in a Shimadu HPLC-DAD system, using an ACE 5C18 column. The antioxidant activity of the extracts was determined by the DPPH and ABTS assays.**
- **A central composite design was performed to determine the effect of three factors (extraction temperature, solvent volume-leaf mass ratio and solvent-to-water ratio) on TPC in extracts, in order to optimize the extraction process.**

EXPERIMENTAL RESULTS

Table 1. Hydroxytyrosol (HT), oleuropein (OP), total phenolic content (TPC) and antioxidant activity in extracts obtained by solid-liquid extraction of olive leaves using several solvents

HT = Hydroxytyrosol
OP = Oleuropein

TPC = Total phenolic content
GAE = Gallic acid equivalent
DM = Dry matter

				Antioxidant activitiy					
				mM Trolox/L		µM Trolox/g DM		% Inhibition	
Solvent	HT (mg/kg DM)	OP (mg/kg DM)	TPC (mg GAE/L)	DPPH	ABTS	DPPH	ABTS	DPPH	ABTS
Hexane	-	-	30.17	0.074	0.173	0.094	0.218	6.56	12.34
Ethyl acetate	-	831.04	926.28	2.645	5.439	0.067	0.137	5.03	8.24
Methanol	56.18	2135.03	1182.39	16.438	23.810	0.415	0.601	24.74	31.75
Ethanol	44.12	3110.39	1324.98	17.213	29.581	0.435	0.747	25.85	39.13

- **Best results, with a high TPC and antioxidant activity, and also a high hydroxytyrosol and oleuropein content in the extracts, were obtained when ethanol and methanol were used as solvents.**
- **The main components present in olive leaf extracts are the phenolic compounds and their derivatives. However, there are many other compounds not yet identified.**

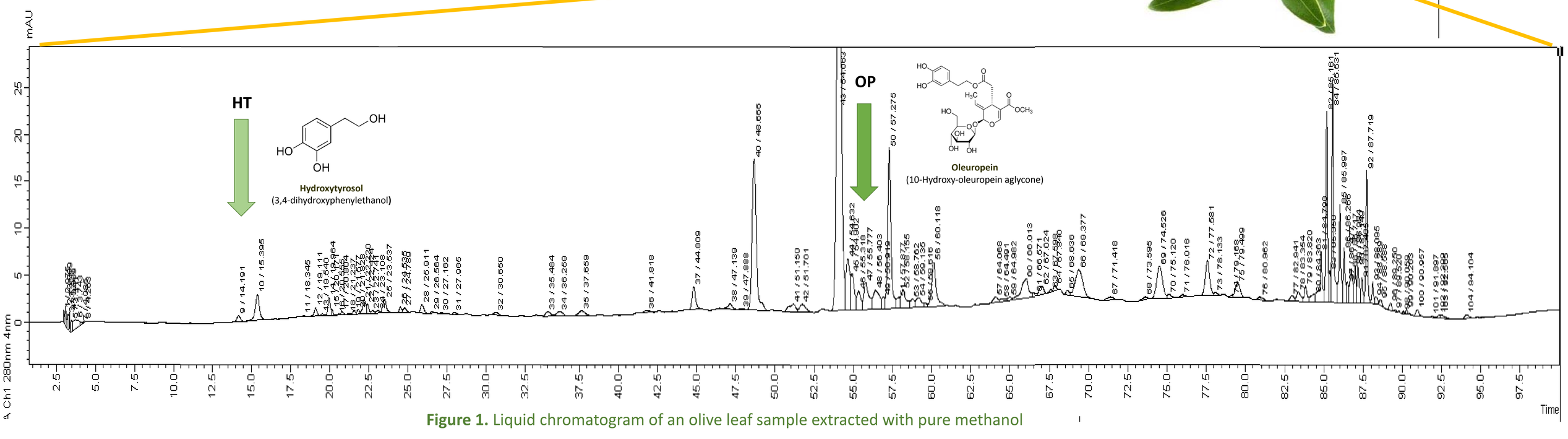

Figure 1. Liquid chromatogram of an olive leaf sample extracted with pure methanol

- **Experimental design using a 2^3 + *star* central composite design (17 experimental runs, with three replicates at the central point) with three levels of each independent variable was performed using methanol as selected solvent to determine the effect of extraction temperature (T = 25-40 ºC), solvent volume-leaf mass ratio (R = 5-15 mL/g) and methanol-to-water ratio (Methanol = 50-90 vol. %) on total phenolic content (TPC) in the extracts as the response variable, in order to optimize the extraction process. The experimental design and data analysis were performed using Statgraphics Centurion XVII software.**
- **Experimental results indicate that, for the range of factors studied, methanol-to-water ratio has the main influence, being statistically significant (p-value < 0.05), on TPC. The model obtained was robust and explains the 87.5% of the variability in TPC (R^2 = 0,875).**
- **A TPC optimum value of 37.19 g GAE/kg DM was obtained for the following operating conditions (Fig. 2): 90 vol.% methanol, T = 32 ºC and solvent volume-leaf mass ratio of 10.6 mL/g.**

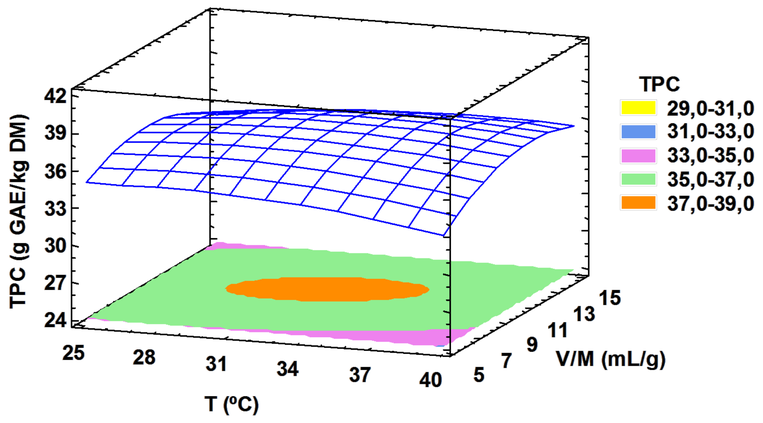

Figure 2. Response surface and contour plots of TPC *vs.* temperature and solvent volume-leaf mass ratio using 90 vol.% methanol as solvent

CONCLUSIONS

- **Phenolic compounds recovery from olive leaves was performed by solid-liquid extraction. Best results, with a high total phenolic content (TPC) and antioxidant activity, and also a high hydroxytyrosol and oleuropein content in the extracts, were obtained when ethanol and methanol were used as solvents.**
- **Experimental design using methanol as solvent was performed to determine the effect of temperature, solvent volume-leaf mass ratio and methanol-to-water ratio on TPC in the extracts. The model obtained explains the 87.5% of the variability in TPC, being methanol-to-water ratio the only statistically significant factor.**
- **A TPC optimum value of 37.19 g GAE/kg DM was obtained for the following operating conditions: 90 vol.% methanol, T = 32 ºC, and solvent volume-leaf mass ratio of 10.6 mL/g.**

ACKNOWLEDGMENTS

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