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Application Notes

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FAST ANALYSIS OF ANTIOXIDANTS IN ALIMENTARY OILS

empresa innovadora de base tecnológica reconocida por ANCES

Fast analysis of antioxidants in alimentary oils

» INTRODUCTION

Determination of **antioxidants** in alimentary oils, especially in olive ones, results of paramount importance to detect product adulterations. Moreover, this phenolic fraction is related to the oxidation resistance, flavor, odor, astringency, etc… of the oil. Tyrosol (TY) and oleuropein (OLEU) are two of the phenolic compounds responsible from these features in olive oils.

Several **separation techniques** have been used for the analysis of antioxidants, such as: gas chromatography (GC) coupled with mass spectroscopy (MS) detector, HPLC-MS and capillary electrophoresis (CE) with MS, ultraviolet (UV) or electrochemical detectors (ED). CE, in its miniaturized format coupled with ED detection present several advantages, against the above mentioned techniques, such as high selectivity and sensitivity, fast analysis and low cost. Thus, **microfluidic electrophoresis systems** in combination with **electrochemical detection** open the gate to new analytical methods for quality control of food.

A ready-to-use portable microfluidic system (MicruX® iHVStat) can be a powerful solution for using **microchips electrophoresis** (ME) and electrochemical detection in the analysis of different alimentary oil samples (virgin **olive oil** (VOO) and **sunflower oil** (SFO)). Thus, the adaptation of a novel analysis methodology enables the separation and detection of antioxidants (TY and OLEU) in virgin olive oil (VOO) and sunflower oil (SFO) in less than 50s preceded by a fast and simple sample pre-treatment.

» EXPERIMENTAL

Samples: Standard solutions of tyrosol and oleuropein. Commercial extra virgin olive oil and sunflower oil.

Sample volume: <100 pL.

Instrumentation: MicruX® HVStat / iHVStat. Holder DC series.

Microfluidic device: SU8/Pyrex microchips with integrated Pt electrodes (*MCE-SU8-Pt001T*).

Conditioning: 0.1 M NaOH – 30 min.
Deionized water – 15 min.
Buffer solution – 10 min.

Sample pretreatment: liquid-liquid extraction of the phenols is carried out from a sample of 1mL of oil. 1mL of n-hexane is added to the oil and then extraction is carried out with 1mL of a mixture of of MeOH:H_2O (3:2, v/v). The MeOH:H_2O layer is separated and this operation is repeated twice. Aqueous phases are combined and heated in a boiling water bath until dryness. Solid extract is dissolved in 500 µL of a mixture of MeOH:H_2O (1:1, v/v). A scheme of that procedure is showed in ***Figure 1***.

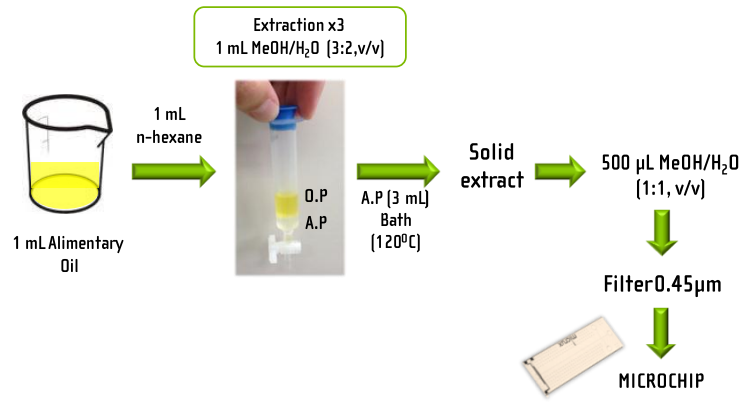

Figure 1. *Off-chip pretreatment of the alimentary oil samples*

» RESULTS & DISCUSSION

Microfluidic electrophoresis system has been used in the separation and detection of tyrosol (TY) and oleuropein (OLEU).

Different separation and detection parameters were studied, obtaining the separation of a mixture of these two standard compounds in less than 50 seconds under optimal conditions (***Figure 2***). The analytical parameters for this methodology are summarized in ***Table 1***.

TY concentration in olive oils rage from 1,9 to 27,5 mg/kg (25 µM) oil and OLEU concentration is directly related with the olive variety used, for example for *arbequina* oils, its concentration is close to 3 mg/ kg oil (20 µM).

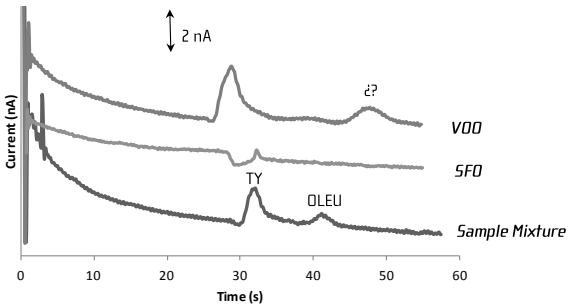

Figure 2. *Electropherograms for a sample mixture of 100 µM TY and 100 µM OLEU and real samples of alimentary oils VOO and SFO. Conditions: V_{inj} = +750 V, t_{inj} = 5 s, V_{sep} = +1000 V, E_d = +0.9 V (vs. Pt) and 100 mM TRIS-Boric, pH =9.0 as running buffer.*

Table 1. *Analytical parameters for the separation of a standard mixture of 100 µM TY and 100 µM OLUE.*

	TY	OLEU
» Repeatability i_p (RSD %)	4%	3%
» Repeatability t_m (RSD %)	1%	1%
» Theoretical plate number (N|m^{-1}):	30.000 ± 2.000	38.000 ± 8.000
» Resolution (R_s):	1,50 ± 0,04	
» Linear range (µM):	10 - 200 (n = 8)	15 - 200 (n = 6)
» Sensitivity (pA·μM^{-1}):	22	24
» LOD (µM)*:	7	7

**Limit of detection considers a signal-to-noise ratio, S/N = 3.*
Conditions: 100 mM TRIS-Boric, pH =9.0, V_{inj} = +850 V, t_{inj} = 3 s, V_{sep} = +900 V, E_d = +0.9 V (vs. Pt).

According to the LOD obtained for TY and OLEU, the methodology proposed can be applied to the determination of these compounds in olive oils.

VOO and SFO samples were analyzed in the ME under optimal conditions (***Figure 2***), and the signal obtained was compared with a sample mixture of TY and OLEU for the identification of the compounds.

TY was detected for VOO whereas its concentration in SFO is negligible. A second peak was detected for VOO; however, the compound has not been identified yet. Three samples of VOO were evaluated using the *standard additions method* in order to avoid any matrix effect and get a better precision (***Figure 3***, ***Table 2***).

For the commercial VOO analyzed, the TY concentration in the sample was 6,2 ± 0,2 mg TY/Kg oil that is in range expected for that compound (1,9 - 27,5 mg/kg oil).

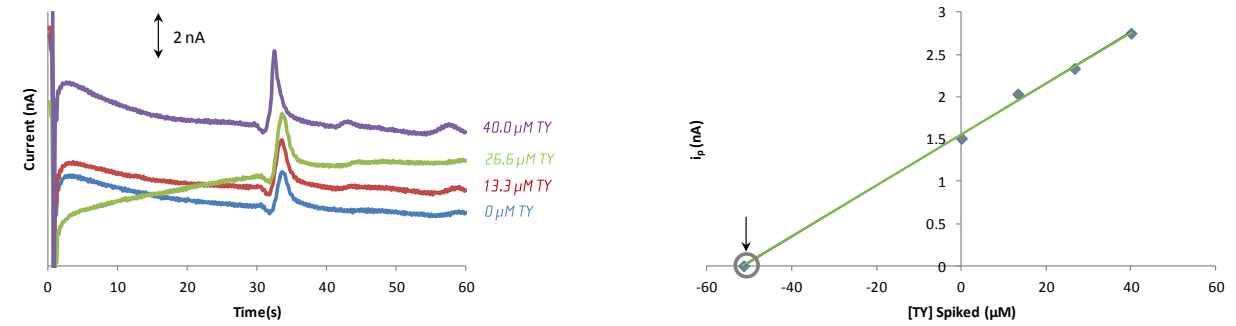

***Figure 3**. **(A)** Electropherograms for a 50 µL olive oil sample with successive additions of TY: 0; 13.3; 26.6 and 40.0µM. **(B)** Standard additions calibration plot. Conditions: V_{inj} = +850 V, t_{inj} = 3 s, V_{sep} = +900 V, E_d = +0.9 V (vs. Pt) and 100 mM TRIS-Boric, pH =9.0 as running buffer.*

***Table 2.** Analysis of TY in VOO samples*

Sample	Slope (nA/µM)	Intercept	TY in VOO (mg/kg oil)
VOO1	0.0302	1.5526	6.08
VOO2	0.0424	2.3350	6.20
VOO3	0.0224	1.2666	6.50

Microchips can be used for two oil sample analysis *per* day, consisting of 20 injections/runs. Microchip must be washed between measures and it is possible to regenerate it from day to day. Thus, it was estimated that the lifetime of a single microchip is close to 1000 experiments following the daily washing protocol.

Therefore, the potential of microchips for the analysis of samples in the agrifood area was demonstrated. A novel methodology for the analysis of antioxidants in oils was proposed including a simplify sample pre-treatment. This methodology results simple, economical, and faster compared with other separation techniques used for the same propose.

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