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FOOD

Application Notes

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Flavones and phenolic acids analysis in MEs-ED

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» INTRODUCTION

Agrofood analysis of raw materials as well as end-products results of paramount importance due to the high market demand and to preserve customer's safety. Final goal consists of ensuring the hygienic, nutritional and sensorial quality of these end-products.

Antioxidants compounds are of great interest in different industry sectors (pharmaceutical, clinical, esthetic, etc.) owing to their specific properties for human health. Flavones and phenolic acids belong to antioxidants family, being part of food and drinks like fruit juices, wine, tea, coffee, etc. Several **strategies** have been used for their analysis, such as: the Folin Ciocalteau method, HPLC and capillary electrophoresis (CE) with different detection systems.

Microchips electrophoresis (MEs) in combination with **electrochemical detection** (ED) open the gate to new analytical methods for quality control of food because of their fast analysis time and low cost. However, **MEs** application for agrofood analysis results complicated due to the matrix nature of the sample affecting sensitivity and selectivity. For that reason, MEs have been used for the separation and detection steps, whereas sample pretreatment is carried out *off-chip.*

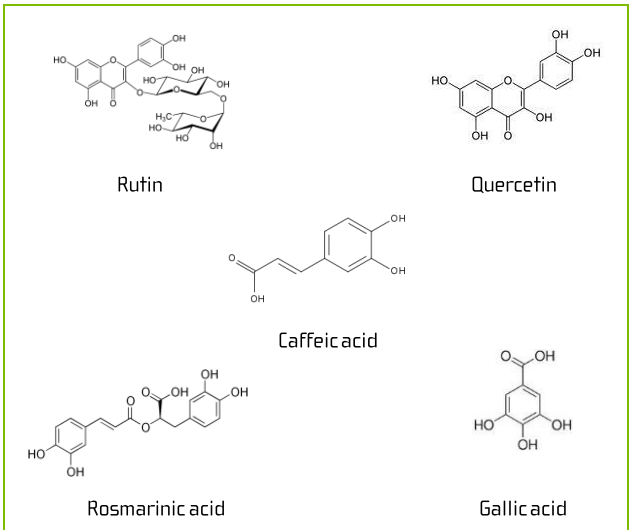

***Figure 1**. Structure of the flavones: rutin and quercetin and the phenolic acids: rosmarinic, caffeic and gallic.*

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 flavones and antioxidants. Thus, a new analysis methodology has been proposed for the analysis of the flavones rutin (RUT) and quercetin (QUER), and the phenolic acids: rosmarinic (RA), caffeic (CA) and gallic (GA) in less than 90 s. (***Figure 1***)

» EXPERIMENTAL

Samples: Standard solutions of rutin, quercetin, rosmarinic acid, caffeic acid and gallic acid.

Sample volume: <100 pL.

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

Microfluidic device: SU8/Pyrex microchips with integrated Pt interdigitated microelectrodes (*MCE-SU8-Pt005T*).

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

» RESULTS & DISCUSSION

Microfluidic electrophoresis system has been used in the separation and detection of the flavones rutin (RUT) and quercetin (QUER), and the phenolic acids: rosmarinic (RA), caffeic (CA) and gallic (GA).

A ME with an interdigitated electrode detector (IDA) design have been used in two different modes: applying the same detection potential over the two working electrodes (*mode 1)* and applying an oxidation potential on WE1 and a reduction one on WE2 (*mode 2*).

A sample mixture consisting on RUT 50 µM, QUER 100 µM, RA 100 µM, CA 100 µM and GA 100 µM has been analyzed. For the injection, separation and detection of these compounds, an injection voltage of +850 V was applied during 5s and the separation voltage was +900 V. Working in *mode 1*, same

detection potential was applied over both WEs ($E_{D(WE1)}$= $E_{D(WE2)}$= +0.9 V). Meanwhile, in *mode 2*, different detection potentials are selected for WE1 and WE2 ($E_{D(WE1)}$= +0.90 V, $E_{D(WE2)}$= -0.25 V).

In ***Figure 2,*** electropherograms obtained for the separation of a sample mixture of the five antioxidants in a microchip with an integrated interdigitated electrode working on *mode 1* are shown. Same signals have been registered over both WEs with negligible differences in peak currents. Peak currents expressed as the addition of the signals registered in both WEs were: 2.67±0.03 nA for RUT and QUER, 1.36±0.05 nA for RA, 1.26±0.05 nA for CA and 1.42±0.01 nA for GA.

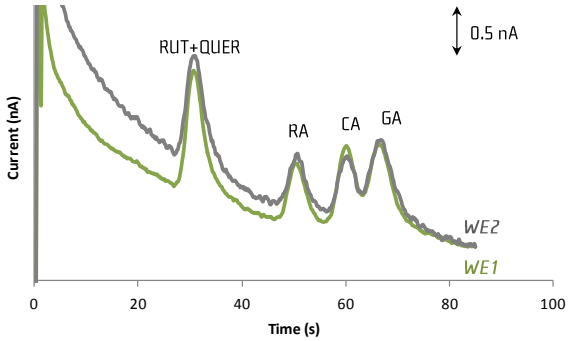

Figure 2*. Electropherograms for a sample mixture of 50 µM RUT and RA and 100 µM QUER, CA and GA. Conditions: V_{inj} = +850 V, t_{inj} = 5 s, V_{sep} = +900 V, $E_{D(WE1)}$= $E_{D(WE2)}$= +0.09 V (vs. Pt) and 20 mM MES-NaOH, pH =5.0 as running buffer.*

Flavones RUT and QUER are neutral compounds in the buffer solution selected (pk_a 6.74 and 6.62 respectively) and they cannot be separated. For that reason, just one peak is observed for the two compounds with a migration time of 32s. This peak represents the total flavones of the sample and it has been used for the determination of the electroosmotic flow (EOF) ((3.25±0.01)x10^{-4} cm^2 V^{-1} s^{-1}). Separation of phenolic compounds (anionic) RA, CA and GA, was possible in less than 80s.

The same sample mixture was analyzed without changing the ME, but applying different detection potentials (*mode 2*) at WE1 and WE2 ($E_{D(WE1)}$= +0.90 V, $E_{D(WE2)}$= -0.25 V). The electropherograms obtained are shown in ***Figure 3.*** Injection and separation voltage and injection time were the same used in *mode 1*.

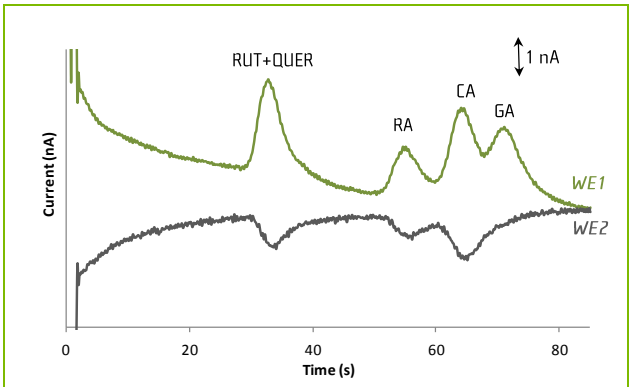

Figure 3*. Electropherograms for a sample mixture of 50 µM RUT and RA and 100 µM QUER, CA and GA. Conditions: V_{inj} = +850 V, t_{inj} = 5 s, V_{sep} = +900 V, $E_{D(WE1)}$= +0.90 V; $E_{D(WE2)}$= -0.25 V (vs. Pt) and 20 mM MES-NaOH, pH =5.0 as running buffer.*

Under these conditions, the oxidation of the five compounds were registered over WE1, whereas in WE2, the reduction of the flavones and the phenolic acids RA and CA that have been oxidized in WE1 were detected.

Peak current measured over WE1 is: 0.88±0.01 nA for the total flavones (RUT+QUER), 0.39±0.01 nA for RA, 0.54±0.01 nA for CA and 0.32±0.02 nA for GA. These oxidation peak currents are lower compared with the ones obtained for *mode 1,* where oxidation peak current correspond to the addition of the signal registered in both WEs. The use of an interdigitated electrode detector system enables the simultaneous determination of oxidation and reduction process of different compounds

Therefore, microfluidic electrophoresis chips are a promising tool for the analysis of samples in the agrifood area. It has been demonstrated the flavones and phenolic acids analysis could be performed in MEs although this methodology is not optimized yet. This analysis will result simple, economical, and faster compared with other separation techniques used for the same propose.

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