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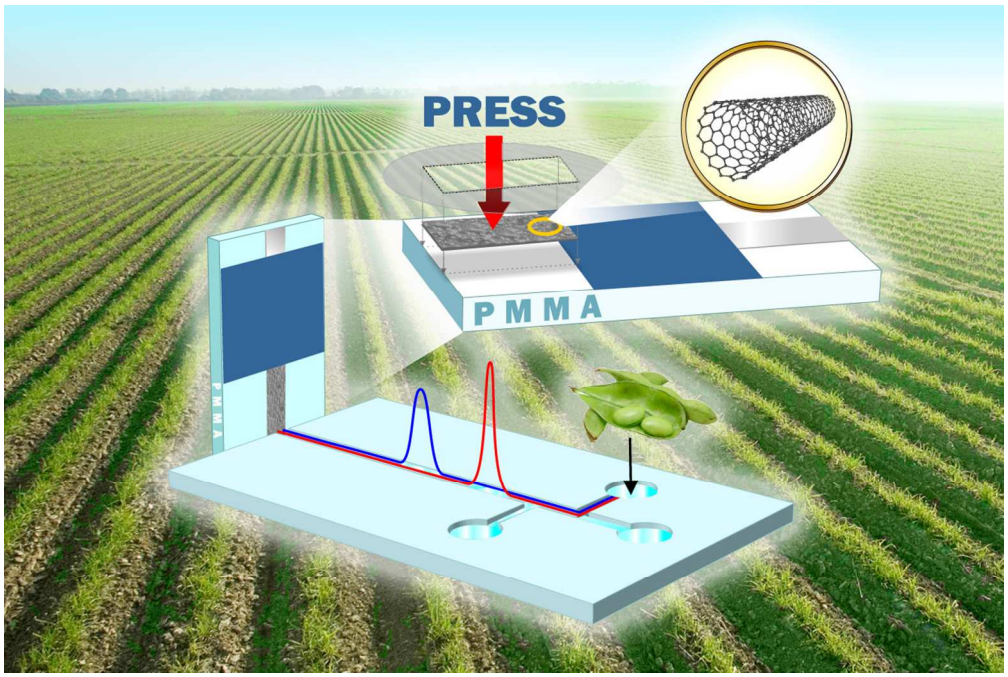

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Direct microfluidic electrochemical sensing of class-isoflavones in complex soy samples on press-transferred carbon nanotubes

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ARTICLE

Fast and reliable class-selective isoflavone index determination on carbon nanotube press-transferred electrodes using microfluidic chips

D. Vilela, A. Martín, M. C. González and A. Escarpa*

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Single-walled carbon nanotube press-transferred electrodes (SWPTEs) are new disposable electrodes where carbon nanotubes act as exclusive electrochemical transducer, being an excellent alternative to common approaches in the field. In the current work, these pioneering SWPTEs coupled to microfluidics chips (MCs) have been employed to develop their first real application. A class-selective electrochemical isoflavone index determination has been proposed for fast and reliable qualitative and quantitative assessment of class-isoflavone based on the co-migration of the total glycosides (TG) and total aglycones (TA) in less than 250 s with very good intra-SWPTEs repeatability (RSDs≤8%, *n=5*) and inter-SWPTEs reproducibility (RSDs≤9%, *n=3*). These novel SWPTEs are entering with important roles into the micro and nanotechnologies scenes expanding new frontiers in the food analysis and health field.

Keywords: Microfluidic chips, carbon nanotubes press-transferred electrodes, aglycones and glycosides isoflavones.

Introduction

Relevant literature has recently demonstrated that microfluidic meets nanomaterials making the micro and nanotechnologies a good combination with plenty of analytical possibilities[1-3]. Electrochemistry on the board of detection for microfluidics has been proven as an ideal and valuable analytical technique due to its inherent facility for miniaturization without loss of performance, high sensitivity and extremely high compatibility with the micro- and nanotechnologies. In this way, one relevant example is the coupling of carbon nanotubes (CNTs) as electrochemical detectors in microfluidic chips (MCs), which has enhanced the analytical performance in terms of selectivity, sensitivity and reproducibility [4-6]. In addition, CNTs-based detectors have offered high heterogeneous electron transfer rate between the analyte and the electrode surface, which results in sharp and less tailing peaks and consequently in a high analyte resolution[1,6,7].

Commonly, the building of CNTs-based detectors for microfluidic sensing has been carried out using two approaches[1]: (i) Thin-film coating and (ii) building CNTs/polymer composites. The design of CNT thin film electrodes is very simple. The underlying bulk electrode is modified with carbon nanotube film, usually by deposition of a CNT suspension in the solvent and allowing its evaporation. The CNTs, consequently, lay randomly as CNT films on the electrode surface. The advantage of this design is the simplicity of the CNT film electrodes and the ability to choose from a wide range of underlying electrodes. Modification by thin film coating has been a useful approach; however, such films are sometimes mechanically fragile being the composite electrodes a good alternative since these detectors are mechanically stable and they significantly reduce the noise levels.

Alternatively to those approaches, recently, we have proposed novel press-transferred carbon nanotubes electrodes (SWPTEs) on poly-methyl-methacrylate (PMMA) substrates for electrochemical microfluidic sensing[8]. In the approach showed in **Figure 1A**, CNT films obtained by filtering of CNT homogeneous dispersions are transferred to PMMA substrate by pressure adopting the electrode geometry suitable for its use as detector in MCs. Basically, SWPTEs have important advantages such as (i) the electronic transfer is carried out directly on the target nanomaterial wherein CNTs are the exclusive transducer avoiding any influence of the conducting support of the CNT film on the electrochemical response, (ii) they can be fabricated from commercial sources of CNTs using a simple protocol which could be afforded in any laboratory, and (iii) they are well-matched with mass-production, disposability and other nanomaterials and/or biological material.

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On the other hand, isoflavones are extremely significant compounds which exhibit interesting antioxidant properties being genistein, daidzein and their related glycosides genistin and daidzin the most widely distributed throughout the plant kingdom, especially in leguminous plant family. Epidemiological studies have indicated that the consumption of soybeans may be associated with a reduction in the risk of cancer, cardiovascular diseases and osteoporosis and they can also be used for the treatment of the symptoms of menopause being also active antioxidants[9].

Analytical determination of isoflavones in soy products has been carried out through two main approaches. One of these approaches has been the determination of the total amount of the isoflavones but not of the different conjugates by releasing aglycones obtained after hydrolysis or saponification reactions. Several analytical methods including spectrophotometry[9], capillary electrophoresis[10], immuno-analysis[11], liquid chromatography[12], gas chromatography[13] and thin layer chromatography[14] have been used with this purpose. Alternatively, the second approach is based on the simultaneous determination of aglycones and their corresponding glycosides in soy products using commonly HPLC. Several HPLC methods for determining isoflavone aglycones and glycosides have been described using UV[15], diode-array (DAD)[16], electrochemical[17] and MS-detection[18]. Likewise, capillary zone electrophoresis coupled with electrospray ionization mass spectrometry (CZE-ESI-MS) has been applied in the separation and quantification of aglycones and glycosides in soy drink[19]. However, only one work using CE with ED has been reported just to separate genistein and daidzein[20]. Finally, CE microchips with ED have also been employed for total isoflavone detection using glassy carbon electrode (GCE)[21] and carbon screen printed electrode (CSPE) casted with CNTs[5].

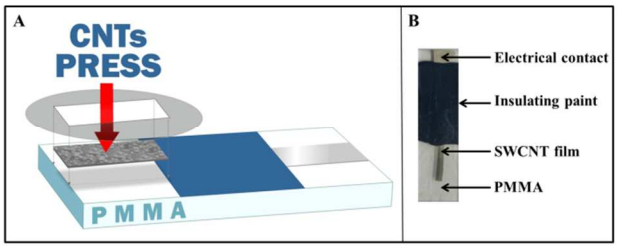

Figure 1. (A) Scheme of the lab-made SWPTE. **(B)** Picture of a lab-made SWPTE.

One important aspect feature of these molecules is that the biological activity and metabolic fate of dietary soy isoflavones vary depending on their chemical form (aglycone or glycoside)[22-25]. As consequence, a fast differentiation of both total aglycones (TA) and total glycosides (TG) content should be very suitable for fast evaluation of antioxidant activity and bio-availability monitorization where the coupling of MC-SWPTEs could be an excellent platform for fast and reliable chemical differentiation and analysis of the TA and TG in the agro-food and health sectors. After the introduction of the proof of the concept[8], in the current work, we propose the first analytical application coupling MC-SWPTEs based on a pioneer determination of TA and TG isoflavones indexes.

Materials and methods

Electrode fabrication

Reagents. Single-walled carbon nanotubes (Sigma-Aldrich), 1,2-dichloroethane (Sigma-Aldrich), poly(methyl methacrylate) (Maniplastic), Teflon filters pore size 0.1 µm (JVWP01300, Millipore Omnipore), silver conductive paint (Electrolube) for the electrical contacts and epoxy protective overcoat (242-SB de ESL Europe) as insulator were used to produce the single-walled carbon nanotubes press-transfer electrodes (SWPTEs).

Electrode fabrication. The electrode fabrication is based on preparing films by filtering homogenized SWCNT dispersions through teflon filters that are transferred to PMMA[8, 26]. Briefly, a stock dispersion of SWCNTs (0.5 mg/100 mL) was prepared in 1,2-dichloroethane, avoiding the use of surfactants. Good dispersions were achieved using an ultrasound bath for 1 hour and a tip sonicator applying a power of 250 W for 5 minutes. Then, 5 mL of the dispersion were filtered under vacuum using a Teflon filter that was dried for 5 minutes. SWCNT films collected on the filter were cut to obtain a SWCNT wire (13 x 1 mm). SWCNT wire was transferred in the middle of PMMA substrate by pressure to a piece (3.4 x 1.0 x 2 mm) of PMMA applying 5 ± 1 tons for 30 seconds. PMMA pieces were cleaned with ethanol and deionized water and then dried, prior to the transference. Teflon filter is removed slowly and carefully using tweezers. SWCNTs are transferred to PMMA providing a very homogeneous film. The electrode geometry was suitable for microfluidic system coupled to *end-channel* into wall-jet configuration. The electrical contacts were made using conductive silver that later is electrically isolated using insulating paint. **Figure 1B** illustrates lab made SWPTE from 5 mL of SWCNT dispersions (0.5 mg/100 mL). As it shown, the detector design for its coupling with the MC consisted of a press-transferred line-film (7×1 mm) positioned and centred on the PMMA substrate (33×9 mm).

Table 1. Isoflavone composition of studied samples.

Isoflavone	Soy Extracts (% isoflavones)		
	A	B	C
Genistein	18.6	0.2	7.5
Daidzein	17.6	2.0	12.7
Total Aglycones (TA)*	36.9	2.6	20.8
Genistin	5.0	6.3	12.6
Daidzin	5.2	24.0	9.1
Total Glycosides (TG)*	11.1	42.0	23.7
Total Isoflavones (TI)	48.0	44.6	44.5

* The total sum of aglycones **(TA)** or glycosides **(TG)** also considers other minority isoflavones.

Microfluidic Chips with Electrochemical Detection

Standards, reagents and samples. Isoflavones standard (daidzein, daidzin, genistein and genistin) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Standard stock solutions of isoflavones were dissolved in EtOH (HPLC grade), to obtain a final concentration of 1.0 x 10^{-2} M for genistein and daidzin and 5.0 x 10^{-3} M for daidzein and genistin, then they were stored at -20ºC. Working solutions were appropriately diluted in borate electrophoresis buffer, purchased from Fluka, and well protected from light and used within 24 h.

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Soy extracts with different composition of isoflavones were provided directly by Exxentia Group (see **table 1**). On the other hand, two dietary supplements (A and B) were acquired in a local pharmacy in Alcalá de Henares (Madrid, Spain). At first, the dietary supplements were mashed and the five different samples were weighed depending on their concentration of isoflavones. Then, the isoflavones were extracted from the samples in absence of light in 20 mL of ethanol using an ultrasonic bath during 30 minutes. The samples were centrifuged at 3000 r.p.m. for 5 minutes and finally, the supernatant was filtered using nylon filters (0.20 µm). The extracts were stored at -20ºC. In all cases water was of high quality, purified in a Milli-Q system (Millipore, Bedford, MA, USA).

Microfluidic chip system. The analytical microsystem set-up was originally reported and described in another work[27] and then adapted[4]. The glass chip was fabricated by Micralyne (Model MC-BF4-001, Edmond, Canada) and consisted of a glass plate (88 mm × 16 mm) with a four-way injection cross, a 74 mm-long separation channel, and side arms measuring 5 mm long. The amperometric detector (end-channel detection) consisted of an Ag/AgCl wire as a reference electrode, a platinum wire as a counter electrode, and a SWCNT electrode (SWPTE) as a working electrode. Amperometric detection was performed using a Potentiostat Autolab PGSTAT12 from Eco Chemie. LabSmith HVS448 High voltage sequencer with eight independent high-voltage channels and programmable sequencing for an entire level of voltage manipulation (LabSmith, Livermore, CA) was used as voltage source.

Microfluidic procedure. The channels of the glass microchip were treated before using and between groups of runs by rinsing them with 0.5 M NaOH for 40 min and deionised water for 10 min. This procedure was carefully monitored to obtain reproducible results. The optimum electrophoresis buffer consisted of 25 mM borate buffer (pH = 9). The running buffer and sample reservoirs were filled with their respective solution. The detection reservoir was filled with 0.1 M nitric acid. A voltage of +1500 V was applied for 5 min to the buffer reservoir to fill the separation channel, while the detection reservoir was grounded and the others were floating. This process was performed to each sample reservoir for 30 s to facilitate filling the injection channel (between the separation channel and the sample reservoir), and then the voltage was applied for 5 min to the running buffer reservoir to eliminate the remains of the previously introduced samples from the separation channel.

Amperometric detection. A detection voltage of +1.0 V was applied to the working electrode for isoflavone analysis. All experiments were performed at room temperature.

Safety considerations. The high-voltage supply should be handled with extreme care in order to avoid electrical shock.

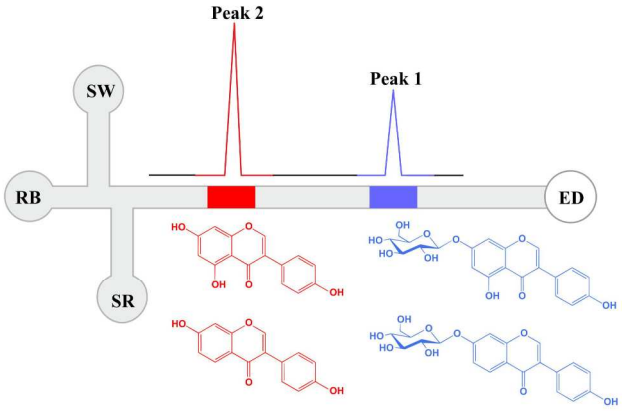

Figure 2. Strategy for the detection of class-selective isoflavone indexes on the microfluidic chip layout. **RB:** running buffer reservoir, **SR:** sample reservoir, **SW:** sample waste reservoir, **ED**: electrochemical detection cell.

Results and discussion

Strategy for the detection of class-selective isoflavone indexes on MC-SWPTEs

Figure 2 illustrates on the MC layout the strategy proposed for the fast assessment of class-selective isoflavone indexes. To this end, the pH of running buffer was strategically established at pH 9.0 (pH> pKa for all isoflavone involved). Under these conditions, both aglycones and glycosides become single ionized and migration of glycosides takes place first (blue peak 1) while the aglycones (red peak 2) migrate afterwards following a classic capillary zone electrophoresis mechanism governed by the molecular sizes. Peak 1 was assigned to the total glycosides (TG) (**Figure 2** illustrates the co-migration of the two main glycosides, genistin and daizdin) and peak 2 was assigned to the total aglycones (TA) (**Figure 2** illustrates the co-migration of the two main aglycones, genistein and daizdein).

Figure 3A shows at this class-selective pH (pH 9), the separation of isoflavone antioxidant classes of glycosides (peak 1) and aglycones (peak 2) in less than 250 s with good resolution (Rs≥1.1) under optimized separation (borate 25 mM, pH 9; +1.5 kV) and detection (E=+1.0 V in 0.1M nitric acid) conditions.

Table 2. Migration times, peak heights and resolutions for each standard mixture.

Isoflavone composition	Peak number	t_m, s[a]	Height, nA[b]	Rs[c]
Genistin	1	147±1	1.9±0.2	1.2
Genistein	2	200±1	3.9±0.1	
Daidzin	1	140±1	2.3±0.1	1.3
Daidzein	2	199±1	4.7±0.1	
Genistin+Daidzin	1	144±3	2.9±0.1	1.1
Genistein+ Daidzein	2	200±3	6.1±0.2	

Expressed as mean values ± standard deviation (n=3). [a]Migration time. [b]Peak height. [c]Resolution.

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Table 3. Analytical features of the calibration graphs.

Analyte	Range (μM)	r	Intercept±tSa[a] (nA)	Slope±tSb[b] (nA μM^{-1})	LOD (μM)
Genistein	50-200	0.990	1.34±1.33	0.030±0.009	20
Genistin	50-200	0.990	0.17±0.89	0.020±0.006	30
Daidzein	50-200	0.994	1.95±0.57	0.030±0.003	20
Daidzin	50-200	0.992	0.25±0.44	0.020±0.003	30

[a] n=3, repeatability in migration time; [b] n=3, repeatability in peak height

In order to demonstrate the viability of the concept, different standard mixtures of target aglycone and glycoside isoflavone-classes were carefully examined: genistin and genistein **(Figure 3 (A)(a))**, daizdin and daidzein **(Figure 3 (A) (b))**, and genistin and daidzin as well as genistein and daidzein **(Figure 3 (A)(c))**. Indeed, the independent analysis of the mixture **(a)** and **(b)** (containing only one glycoside and one aglycone molecules) gave two well-separated peaks corresponding to the migration of the corresponding isoflavone-classes in each case; however, the analysis of a mixture **c** (containing the four target prominent isoflavones) gave also a two-well separated peaks corresponding to the co-migration of both genistin and daizdin (TG) and both genistein and daidzein structures (TA) demonstrating the suitability of the strategy proposed.

The migration times, the peak heights and resolutions for each standard mixture are listed in **Table 2.** The excellent concordance obtained in the migration time confirms the co-migration of the individual glycosides and aglycones showing class-isoflavone well-resolved peaks. The results also showed good intra-electrode repeatability in the migration times (RSD≤2% for glycosides and RSD≤1% for aglycones, n=3) as well as in the peak height (RSD≤8% for glycosides and RSDs≤3% for aglycones, n=3).

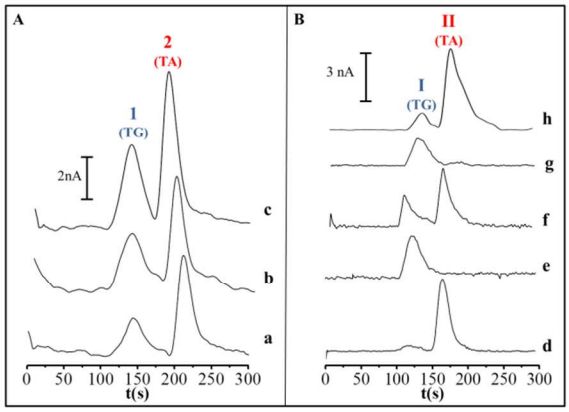

Figure 3. (A) Microfluidic standard isoflavone profiles (100 μM each) using SWPTEs: **(a)** Genistin (peak 1) and Genistein (peak 2), **(b)** Daidzin (peak 1) and Daidzein (peak 2) and **(c)** Genistin and Daidzin (peak 1), Genistein and Daidzein (peak 2). **(B)** Microfluidic isoflavone profiles corresponding to extract A **(d);** extract B **(e)**; extract C **(f)**; dietary supplement (A) **(g)** and dietary supplement (B) **(h)** at SWPTE. Peak I: Total Glycosides (TG); peak II: Total Aglycones (TA). Conditions: Borate buffer 25 mM pH=9, separation voltage +1.5 kV, injection voltage +2.0 kV for 5 s, detection potential +1.0 V (0.1 M nitric acid).

In addition, electrode fabrication reproducibility was also evaluated using different electrodes and evaluating their electro-analytical response. The results obtained showed good inter-electrode reproducibility in the migration times (RSD≤4%, for glycosides and RSD≤7% for aglycones) as well as in the peak height (RSD≤6% for glycosides and RSDs≤9% for aglycones) when three electrodes were employed. This excellent inter-electrode reproducibility indicates the inherent disposability of SWPTE which constitutes an extra advantage of this approach.

Detection and determination of class-selective isoflavone indexes on MC-SWPTEs in soy samples

Table 3 lists the analytical features of the calibration graphs. The resulting calibration plots were highly linear (r≥0.990) in the concentration range assayed (50-200 μM) with higher sensitivities for aglycones (0.030±0.001 nA μM^{-1}) than for glycosides (0.020±0.001 nA μM^{-1}). Taking into account both the sensitivity of the SWPTEs and their noise level, suitable LODs about μM (S/N=3) were obtained. Excellent precision was also obtained with independence of the concentration assayed (RSDs ≤2% and RSDs≤3% for migration times and oxidation currents, respectively).

The qualitative assessment of isoflavone indexes towards detection of TA and TG in soy samples was also explored. **Figure 3B** shows the class-isoflavone profiles for each sample studied: three soy-extracts **(d-f)** with a well-documented and different isoflavone composition (see **Table 1**) and two dietary supplements **(g-h)** with a known nominal value. Two peaks clearly resolved for total glycosides, TG (peak I) and total aglycones, TA (peak II) in less than 250 s were also found during the soy sample analysis (please, note that as more isoflavone structures are also present in soy samples, roman numbers have been used for peak assignment, in order to differentiate them from the standards).

Interestingly, the independent analysis of the extract A containing both TG (11.1%) and TA (36.9%) **(Figure 3B d)**, the extract B containing only a glycoside composition (42.0%) **(Figure 3B e)** and extract C containing both TG (23.7%) and TA (20.8%) **(Figure 3B f),** showed the expected class-isoflavone profile towards the detection of TG and TA in each case. Indeed, two well-separated peaks I and II for extract A and C and a single peak I for extract B were noticed. Also, the analysis of dietary supplement A **(Figure 3B g)** containing only isoflavone glycosides gave their corresponding peak I whereas the other dietary supplement B **(Figure 3B h)** gave the two separated peaks I and II revealing the presence of both TG and TA fractions. This dietary supplement was labelled just with total isoflavone (TI) without differentiation which added a valuable merit to our proposal. Also, an agreement between the migration times obtained for peaks I (140±3 s) and II (182±1 s)

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Table 4. Class-selective isoflavone indexes from soy samples[a]

Sample		Sample composition (% $_{w/w}$)	Obtained value (%$_{w/w}$)	RSD (%)	Error (%)
Extract A	**TA**	36.9	39.3	4	6
	TG	11.1	9.9	3	11
	TI	48.0	49.2	3	2
Extract B	**TA**	2.6	-	-	-
	TG	42.0	44.8	9	6
	TI	44.6	44.8	9	1
Extract C	**TA**	20.8	12.6	13	>15
	TG	23.7	36.7	11	>15
	TI	44.5	49.3	9	11
Dietary supplement A	**TG**	-	5.5	-	-
	TI	5.0	5.5	1	10
Dietary supplement B	**TA**	-	14.9	6	-
	TG	-	18.8	12	-
	TI	30	33.7	8	12

[a] Values are an average of three independent determinations.
TA: Total Aglycones, **TG:** Total Glycosides, **TI:** Total Isoflavones.

in comparison with those obtained for standards was also observed. These results confirmed that all glycoside and all aglycone structures contained in the complex soy extracts co-migrated into the single peaks I and II, respectively.

Table 4 lists quantitatively the class-selective isoflavone electrochemical index obtained from soy samples. With the exception of TA and TG in extract C, a very good agreement between the fractions TA, TG and TI were clearly obtained by the MC-SWPTE presented approach and reference values given in the sample composition. Indeed, low errors were found in all examined cases being of particular attention the lowest obtained (E_r<3%) in the soy extracts in which TA is higher than TG (extract A) or in extracts where TG was the prominent composition (extract B). Also, in the analysis of dietary supplements, a good agreement between the values obtained using MC-SWPTEs approach and the declared value of the manufacturer was also found (E_r≤12%). A good precision was also obtained during the soy sample analysis.

Taking into account the whole results obtained, a class-selective electrochemical isoflavone index determination is proposed to describe the total amount of each isoflavone-class. This index could be very useful for labelling valuable soy samples.

Finally and in comparison with the previous literature stated in the introduction, it could be said that the presented approach is a faster and simpler alternative than the previous ones based on sophisticated instrumentation and toxic solvents. However, this comparison needs to be made carefully since here we propose a novel application for the fast assessment of both isoflavone-classes in contrast with the detailed isoflavone profiles obtained using previous approaches.

More important is that the current work exploits the well-known advantages of the MCs to obtain useful analytical information, becoming them in excellent screening platforms as an alternative to other -sometimes unnecessary- time consuming approaches. In addition, the success obtained in the current novel application reveals the analytical power of the press-transfer technology where the fast and reliable determination of isoflavone indexes was performed directly on the nanoscale carbon nanotube detectors without any other transducer. It allows the possibility to explore directly the advantages of the carbon nanotubes without the need of other sophisticated facilities.

Conclusions

In the present work, the SWPTEs have demonstrated to be a very useful analytical tool for a novel application on the board of MCs such as the fast differentiation of isoflavone chemical forms into glycoside and aglycone structures in soy samples. The qualitative and quantitative assessment was carried out directly on CNTs without necessity of any other transducer material reinforcing the suitability of the MC-SWPTEs coupling where high-sensitive detectors in ultra-small spaces need to be placed. The excellent analytical performance obtained during the analysis of complex soy extracts allowed to demonstrate the reliability of the approach and these novel SWPTEs are entering with important roles into the micro and nanotechnologies scenes expanding new frontiers in the food analysis and health field. These results also add an extra value to the press-transfer technology in the nanotechnology scene as clear alternative to other conventional transducers.

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Notes and references

Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, University of Alcalá, E-28871 Alcalá de Henares, Madrid, Spain, 918854971,* alberto.escarpa@uah.es

(1) Pumera, M.; Escarpa, A. *Electrophoresis*, **2009**, *30*, 3315-3323
(2) Pumera, M. *Chem. Comm.*, **2011**, *47*, 5671-5680.
(3) Escarpa, A. *Chem. Rec.*, **2012**, *12*, 72-91.
(4) Crevillén, A.; Ávila, M.; Pumera, M.; González, M. C.; Escarpa, A. *Anal. Chem.*, **2007**, *79*, 7408-7415.
(5) Crevillén, A.; Martin, P.; González, M. C.; Escarpa, A. *Lab Chip*, **2009**, *9*, 346-353.

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(6) Vilela, D.; Anson-Casaos, A.; Martinez, M. T. ; González, M. C.; Escarpa, A. *Lab Chip*, **2012**, *12*, 2006-2014.
(7) Wang, J.; Tian, B.; Chatrathi, M.P.; Escarpa, A.; Pumera, M. *Electrophoresis*, **2009**, *30*, 3334-3338.
(8) Vilela, D.; Garóz, J.; Colina, A.; González, M. C.; Escarpa, A., *Anal. Chem.*, **2012**, *84*, 10838-10834.
(9) Toro-Funes, N.; Odriozola-Serrano, I.; Bosch-Fusté, J.; Latorre-Moratalla, M. L.; Veciana-Nogués, M. T. Izquierdo-Pulido, M.; Vidal-Carou, M. C., *Food Chem.,* **2012**, *135*, 2832-2838.
(10) Micke, G. A.; Fujiya, N. M.; Tonin, F. G., *Journal of Pharmaceutical and Biomedical Analysis*, **2006**, *41*, 1625-1632.
(11) Dentith, S.; Lockwood, B., *Curr. Opin. Clin. Nutr. Metab. Care*, **2008**, *11*, 242-247.
(12) Apers, S.; Naessens, T.; Van Den Steen, K.; Cuyckens, F.; Claeys, M.; Pieters, L., *J. Chrom. A*, **2004**, *1038*, 107-112.
(13) Ghosh, P.; Fenner, G. P., *J. Agric. Food Chem.*, **1999**, *47*, 3455-3456.
(14) César, I. D. C.; Braga, F. C.; Vianna-Soares, C. D.; Nunan, E. D. A.; Barbosa, T. A. F.; Moreira-Campos, L. M., *Braz. J. Pharmacog.*, **2007**, *17*, 616-625.
(15) Klump, S. P.; Allred, M. C., Macdonald, J. L.; Ballam, J. M., *J. AOAC Int.,* **2001**, *84*, 1865-1883.
(16) Franke, A. A.; Custer, L. J., Cerna, C. M.; Narala, *K. Proc. Soc. Exp. Biol. Med.,* **1995**, *208*, 18-26.
(17) Setchell, K. D. R.; Welsh, M. B., *Br. J. Nutr.*, **1987**, *386*, 315-323.
(18) Wiseman, H. C.; Clarke, D. B.; Barnes, K. A.; Bowey, E., *J. Agric. Food Chem.*, **2002**, *50*, 1404-1410.
(19) Bustamante-Ragel, M.; Delgado-Zamarreño, M. M.; Carabias-Martínez, R.; Domínguez-Álvarez, J.*, Anal. Chim. Acta*, **2012**, *709*, 113-119.
(20) Peng, Y.; Chu, Q.; Liu, F.; Ye, J., *Food Chem.,* **2004**, *87*, 135-139.
(21) Blasco, A. J.; Crevillén, A. G.; de la Fuente, P.; González, M. C.; Escarpa, A., *Analyst,* **2007**, *132*, 323-329.
(22) Cassidy, A., *Pro. Nutr. Soc.*, **1996**, 55, 399-417.
(23) Kelly, G. E.; Nelson, C.; Waring, M. A.; Joannou, G. E.; Reeder, A. Y., *Clin. Chim. Acta*, **1993**, *223*, 9–22.
(24) Xu, X.; Wang, H.-J.; Murphy, P. A.; Cook, L.; Hendrich, S., *J. Nutr.*, **1994**, *124*, 825–832.
(25) Setchell, K. D. R.; Borriello, S. P.; Hulme, P.; Kirk, D. N.; Axelson, M., *Am. J. Clin. Nutr.*, **1984**, *40*, 569 –578.
(26) Garoz-Ruiz, J.; Palmero, S.; Ibañez, D.; Heras, A.; Colina, A. *Electrochem. Commun.,* **2012**, *25*,1-4.
(27) Wang, J.; Tian, B.; Sahlin, E., *Anal. Chem*., **1999**, *71*, 5436-5440.

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