



# High-performance carbon black/molybdenum disulfide nano hybrid sensor for cocoa catechins determination using an extraction-free approach

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## ABSTRACT

In this work, for the first time, the ability to detect catechins on an effective and regenerable carbon black/molybdenum disulfide nano hybrid screen-printed electrode (SPE-CB/MoS<sub>2</sub>) is demonstrated. The proposed SPE-CB/MoS<sub>2</sub> merges the ability of CB to improve the electrochemical response with the properties of MoS<sub>2</sub> to totally prevent catechins irreversible polymerization and adsorption onto the electrode surface occurring at both bare and CB-modified SPEs. The MoS<sub>2</sub> anti-fouling ability has been demonstrated using both flavanols standards and complex samples thus proved to be an unexpected and useful characteristic. The SPE-CB/MoS<sub>2</sub> allowed an improvement of sensitivity (LOD  $\leq 0.17 \mu\text{mol L}^{-1}$ ) of 100-folds compared to the bare SPE electrode, showing linear range between 0.12 and 25  $\mu\text{mol L}^{-1}$  with good determination coefficients ( $R^2 \geq 0.998$ ). Cocoa powder samples have been analyzed employing the developed hybrid sensor and compared with classical methods for polyphenols evaluation as Folin-Ciocalteu (F.C.), ABTS and an AuNPs based assay. The SPE-CB/MoS<sub>2</sub> allow to obtain repeatable and reproducible results which also were significantly correlated with classical methods ( $r = 0.95\text{--}0.97$ ). Noteworthy, after the measurements of 59 cocoa samples the electrode was still active (recovery signal 99%). Using the extraction-free approach with the SPE-CB/MoS<sub>2</sub> sensor the whole analysis of cocoa powder catechins required only 15 min.

## 1. Introduction

Recent trends in food marketing suggest that cocoa and cocoa-products present multiple-benefits which are becoming subject of increased scientific research, mainly because of their phytochemical composition [1,2]. In fact, the high polyphenols content of cocoa has generated a renewed interest for nutritional and health viewpoints [3], and thanks to this properties, the global demand for cocoa is increasing [4]. In the cocoa-based products market, the cocoa content results to be an added value, influencing price and the organoleptic properties [5]. Cocoa is rich in flavanols (catechins) and proanthocyanidins, strong antioxidants, which have been associated with several potential health benefits [1,3]. Moreover, they also affect the cocoa fat resistance towards oxidation, during the production process and conservation [3,6–8]. In fact, the final content of antioxidant compounds in cocoa-

based products is a function of several variables related to the raw material and others related to processing and formulation [3,6–8].

Cocoa catechins contents and their antioxidant capacity evaluation are still a challenging issue, in particular for the complexity of the matrix. Indeed, the extraction step is necessary to remove the possible interfering compounds, separate the polyphenols from the lipids, but can lead to low recovery, resulting in consumption of solvents and time [9]. The cocoa polyphenolic extracts classically are analyzed with spectrophotometric assays or chromatographic methods [2,3,10–14]; the latter can provide identification of the constituents and amount, but do not returns direct information on the antioxidant capacity of polyphenols [15]. Classical spectrophotometric methods are simple but can present some disadvantages as the use of expensive reagents, not environmentally friendly (i.e. radical compounds, metals, etc.), undefined reaction time, long sample pretreatment, and low precision, selectivity,

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and sensitivity [16]. Several other methods have been proposed for polyphenols cocoa analysis, as e.g. capillary electrophoresis [17], NIR [18] and FT-IR spectroscopy [19], and only few methods don't require sample preparation and extraction [9].

Electrochemical sensors represent a valuable alternative approach to the detection of polyphenols and antioxidant capacity [15,16] and different electrochemical approaches for the direct sensing using several electrodes/transducers types and configurations have been proposed [15,16,20–22]. However, direct electrochemical oxidation of polyphenols onto classical unmodified carbon-based materials is affected by the typical drawback of fouling [15,23]. This phenomenon is particularly relevant for flavanols (catechins) structures [24]. In fact, the redox behavior of catechins onto carbon electrodes, despite being initially apparently reversible, results in an electrode surface irreversible passivation [25]. The passivation is caused by adsorption and polymerization of oxidation products onto the working electrode surface [15,23]. This drawback causes loss of sensitivity and reproducibility and drives versus the disposable use of the electrodes. Nanomaterials (NMs) have attracted attention since they can reduce the passivation of the electrode area, potentially improving also sensitivity and selectivity [15,26–30]. Different NMs have been used for catechins direct sensing purposes, as nanotubes [31], graphene [32,33], etc. [24,34–38]. However, the catechins by-products of oxidation tendency to interact with the electrode surface is strong and this does not allow reproducible and accurate analysis. In fact, very often the electrochemical behavior of the catechins is studied as proof of concept, without a deep study of both passivation and exploitability with complex samples [15]. This drawback and the need of an extraction step have limited the use of electrochemical sensors in this field, for example, as very few application of sensors on cocoa samples are reported in literature [35,38].

In the last years, carbon black (CB) has been widely employed for building electrochemical platforms. Its particular features stimulated intense research efforts; in fact, CB nanospheres possess high surface area, high electrical conductivity, low cost, and high dispersibility and can be easily prepared for the realization of electrochemical sensors [15,39–42]. On the other hand, transition Metal Dichalcogenides (TMDs) materials have become an attractive alternative for electrochemical applications [43]. Layered transition-metal dichalcogenides, in particular,  $WS_2$  and  $MoS_2$ , have attracted increasing interest as modifiers of working surfaces for hydrogen evolution reaction (HER) [44,45] and energy storage [46–48]. The use of  $MoS_2$  in electrochemical sensing is a growing field and holds great promise in sensors and biosensors development [49–51]. However, despite the carbon black have been used for the construction of some electrodes for the direct polyphenol detections [15,52], even in foods matrices as olive oil [53], the potential of the use of  $MoS_2$  in the direct detection of polyphenols has not been yet exploited; just a paper reported on the  $MoS_2$  as electrode modifier for laccase biosensor used for red wine polyphenols indirect estimation [54].

In the last years  $MoS_2$ -based nanocomposites have attracted researchers' attention [49]; since some  $MoS_2$  hybrid materials have shown great versatility as advanced electrode modifier, showing unique and sometimes unexpected sensing properties and catalytic activity [51,55,56]. The integration of carbon-based NMs [54,57], metal [58] or metal oxide nanoparticles [56] in  $MoS_2$  structures has been reported to have synergistic effects, exploitable in the electrochemical sensing.

The present paper describes, for the first time, the catechins determination ability on a new CB/ $MoS_2$  nanohybrid electrode (SPE CB- $MoS_2$ ). The analytical performance has been tested together with the regenerability of the sensor. The developed sensor was then applied to the assessment of the catechins content of 59 cocoa powder samples, expressed as epicatechin equivalent. The results obtained for the 59 cocoa powder samples correlated well with classical methods as Folin-Ciocalteu (FC), ABTS and gold nanoparticles-based (AuNPs) assay.

## 2. Materials and methods

### 2.1. Reagents and stock solutions

All the chemicals were of analytical grade.  $MoS_2$ , Epicatechin, catechin, epigallocatechin, cetyltrimethylammonium chloride (CTAC; 25% in water), hydrogen tetrachloroaurate ( $HAuCl_4 \cdot 3H_2O$ , 99.9%), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), sodium carbonate ( $Na_2CO_3$ ), Folin & Ciocalteu's phenol reagent, sodium phosphate monobasic monohydrate ACS reagent ( $NaH_2PO_4 \cdot H_2O$ ) sodium phosphate dibasic anhydrous ( $Na_2HPO_4$ ), potassium ferrocyanide, potassium ferricyanide, potassium chloride, methanol, *N,N*-dimethylformamide, dimethyl sulfoxide (DMSO), acetonitrile, acetic acid, sodium acetate, formic acid were purchased from Sigma Aldrich (St Louis, MO, USA). CB N220 was obtained from Cabot Corporation (Ravenna, Italy). Polyphenol stock solutions were prepared in methanol at a concentration of  $1.0 \times 10^{-2} \text{ mol L}^{-1}$  and stored in the dark at  $-20^\circ\text{C}$ . Milli-Q water (18.2 M $\Omega$ ) was employed for all the experiments.

### 2.2. Apparatus

Electrochemical measurements were carried out using an Autolab PGSTAT 12 potentiostat from Metrohm (Utrecht, Netherlands), screen-printed electrodes (SPE) were purchased from Dropsens S.L. (ref. DS110). Samples were shaken and centrifuged with an SSL1 orbital shaker from Stuart equipment (Belfast, UK) and an ALC4237R refrigerated centrifuge (ALC Intl., Cologno Monzese, Italy), respectively. For the gold nanoparticles assay, the reaction was performed in a water bath using a 720 D thermostat digital group (Asal, Italy). Absorbance was recorded using a JENWAY 6400 spectrophotometer from Barlworld Scientific (Staffordshire, UK).

### 2.3. Samples

A large number ( $n = 59$ ) of cocoa powder commercially available samples were analyzed. The investigated samples, coming from different varieties of cocoa and subjected to different production processes, were codified with numbers from 1 to 59 for reasons of corporate confidentiality. The samples were stored in plastic bags with pressure closure and kept in a cool, dry and dark place to avoid degradation phenomena.

In order to speed up the analysis, cocoa catechins were directly solubilized in DMSO, according to Della Pelle et al. 2015 [9], with some modifications. Briefly, 0.1 g of cocoa powder were weighted and solubilized in 1.5 ml of DMSO. The dispersion was vortexed for 1 min and sonicated in an ultrasonic bath for 5 min at a temperature of  $20^\circ\text{C}$ . Subsequently, the dispersion was centrifuged at 12,000 rpm for 5 min at a temperature of  $+4^\circ\text{C}$  for 10 min. The resulting supernatant was recovered and stored at  $-20^\circ\text{C}$  in the dark until analysis. In order to prove the effectiveness, of the proposed sample pretreatment, the samples have been extracted even according to Gu et al. [59], in this case, the defatting step [8] is followed by a solid-liquid extraction [59]. Both extracts, prior to each further analysis, were diluted to the appropriate dilution in  $0.01 \text{ mol L}^{-1}$  (pH 7.0) phosphate buffer (PB).

### 2.4. Preparation of the SPE-CB/ $MoS_2$

CB and  $MoS_2$  dispersion volume, ratio/concentration and solvent were optimized in order to obtain the most effective electrodes in terms of sensitivity and reproducibility. The  $1 \text{ mg mL}^{-1}$  CB and  $MoS_2$  dispersions were separately prepared in DMF-H<sub>2</sub>O 1:1 (v/v) using a bath sonicator (3000683 Ultrasons Selecta, Barcelona, Spain) for 5 h at a temperature below  $20^\circ\text{C}$ . The CB- $MoS_2$  75:25 (v/v) dispersion was prepared by mixing CB and  $MoS_2$  dispersions, the mix was further sonicated for 1 h. This dispersion results usable for 1 month, however, before the use it requires a further 1 h of sonication. This solution was

employed for the SPEs modification, performed via drop casting onto the working electrode surface in five steps, for a total of 10  $\mu\text{L}$  allowing drying and solvent evaporation after each deposition, the latter process was sped up thanks to a warm light lamp. Before use, the electrodes were pretreated using CV (10 scans) in 0.1  $\text{mmol L}^{-1}$  PB (pH 7) in 0.1  $\text{mol L}^{-1}$  KCl, in the  $-0.30 \text{ V} / +0.7 \text{ V}$  potential range (vs. pseudo-Ag/AgCl), using a scan rate of  $0.5 \text{ V s}^{-1}$ . This procedure was also employed for the electrode surface regeneration, after the measurement of catechins standards or samples.

## 2.5. Morphological characterization

Field Emission Scanning Electron Microscopy (FE-SEM) was used to investigate the morphology of the SPE-CB/MoS<sub>2</sub> sensors. All the experiments were performed with a SIGMA high-resolution scanning electron microscope (Carl Zeiss Microscopy GmbH, Germany). Measurements were conducted on uncoated samples with an acceleration potential of 2 kV and at a working distance of about 4 mm. All the micrographs were analysed with an image processing software (ImageJ, v.1.49.p) [60] in order to evaluate the mean dimension of the MoS<sub>2</sub> crystals and the CB particles, together with the homogeneity of surface coverage and the relative percentage of MoS<sub>2</sub> and CB in the SPE-CB/MoS<sub>2</sub> electrode.

## 2.6. Electrochemical measurements (CV, EIS, and DPV)

The SPEs electrochemical behavior was investigated using CV and DPV; the measurements were carried out by deposition of 100  $\mu\text{L}$  of the analytes solution on the sensor surface. CVs of analytes were carried out using a scan rate of  $0.050 \text{ V s}^{-1}$  in the potential range  $-0.20 \text{ V} / +0.70 \text{ V}$  (vs. pseudo Ag/AgCl) at different pHs: 4.0 and 5.0 in acetate buffer (0.01  $\text{mol L}^{-1}$ ) and pH 6.0, 7.0, 8.0, 9.0 and 10.0 in phosphate buffer (0.01  $\text{mol L}^{-1}$ ). The electrode passivation, after catechins analysis, was further assessed by electrochemical impedance spectroscopy (EIS) using 5  $\text{mmol L}^{-1}$  Fe(CN)<sub>6</sub><sup>4-/3-</sup> in 0.1 M KCl. A sinusoidal wave with an amplitude of  $\pm 10 \text{ mV}$  was employed, in a frequency scan range  $10^{-2}$ – $10^5 \text{ Hz}$  at open circuit potential, the results were analyzed by Nyquist plot ( $-Z_{re}$  vs.  $Z_{im}$ ). DPV was used to quantify the analytes, the best DPV conditions were found using the simplex method, with the final aim to maximize the signal/noise ratio. DPVs were performed in phosphate 0.01  $\text{mol L}^{-1}$  PB (pH 7.0), with a potential range of  $-0.15$  to  $+0.35 \text{ V}$  with a pulse width of 50 ms, and the final DPV pulse amplitude and scan rate were 20 mV and  $50 \text{ mV s}^{-1}$ , respectively. Before the use, the electrodes were pretreated with CVs (5 scans) in 0.1  $\text{mmol L}^{-1}$  PB (pH 7) in 0.1  $\text{mol L}^{-1}$  KCl, in the potential range of  $-0.30 \text{ V}$  and  $+0.7 \text{ V}$  (vs. pseudo-Ag/AgCl), using a scan rate of  $0.5 \text{ V s}^{-1}$ . The latter procedure was also employed for the electrode surface regeneration, after the measurement of catechins standards or samples.

## 2.7. Methods comparison

In order to compare the data obtained with the proposed sensor, the 59 cocoa powder samples were analyzed with three conventional methods for the polyphenols evaluations: ABTS, F.C., and AuNPs. Epicatechin standard solutions were used as reference standard to calibrate the methods. For each method, the possible DMSO reactivity/perturbation has been evaluated, however, there were no significant changes with respect to the aqueous solvent (at the dilution employed for the sample analysis).

### 2.7.1. Folin-Ciocalteu procedure

20  $\mu\text{L}$  of a properly diluted cocoa sample, extracted in the conventional way and with the DMSO strategy (Section 2.3), was added to 20  $\mu\text{L}$  of Folin-Ciocalteu reagent and stirred for 3 min. Then, 400  $\mu\text{L}$  of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>, 7.5%) and deionized water were added up to the final volume of 1000  $\mu\text{L}$ . The solution was stirred at room

temperature for 60 min, in the dark, and the total polyphenol content was determined at 760 nm.

### 2.7.2. ABTS assay procedure

ABTS reagent stock solution was prepared according to Re et al. 1999 [61], the resulting radical ABTS<sup>•+</sup> solution was diluted to reach an absorbance, reading at 734 nm, of 0.70 ( $\pm 0.02$ ). An appropriate diluted cocoa extract volume, extracted in the conventional way and with the DMSO strategy (Section 2.3), was added to the ABTS reagent (final volume 2000  $\mu\text{L}$ ). Then the reaction mix was stored at room temperature for 5 min, in the dark, to evaluate the radical scavenging activity. The absorbance value at 734 nm was recorded and the sample mediated ABTS<sup>•+</sup> shutdown was evaluated with respect to the blank/control, prepared without the sample addition (using the same amount and type of solvent used for extraction) with the following equation: absorbance decrease (%) = [(control absorbance value - sample absorbance value) / control absorbance value]  $\times 100$ .

### 2.7.3. Gold nanoparticles assay procedure

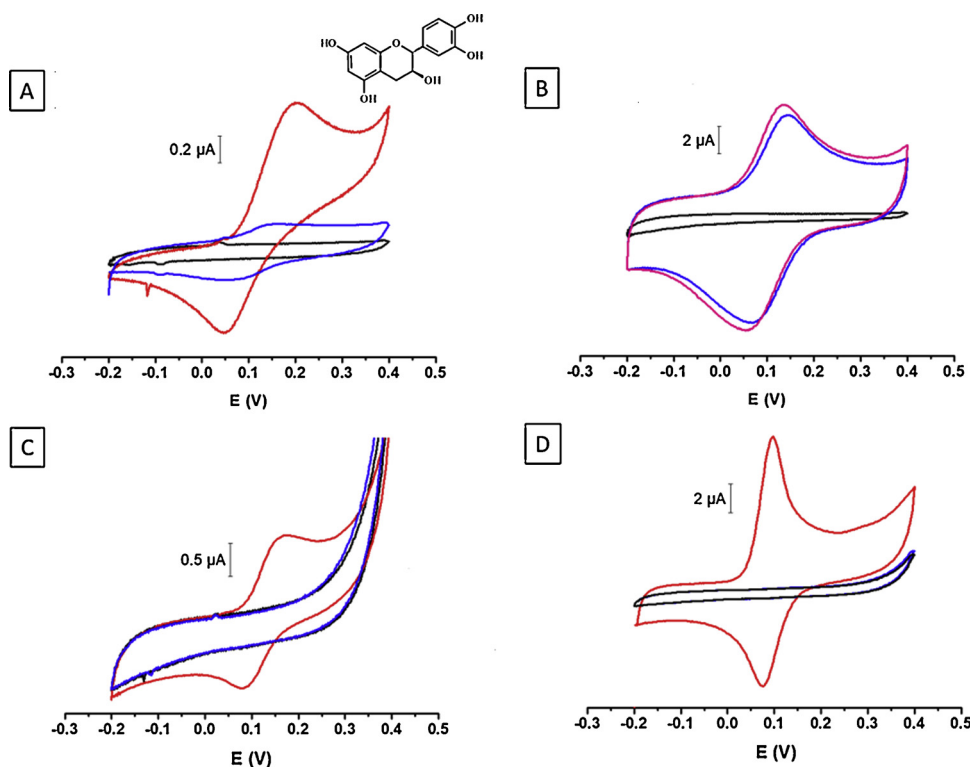
AuNPs based colorimetric assay was used for the total polyphenols determination according to Della Pelle et al. 2015 [9]. For the AuNPs formation 30  $\mu\text{L}$  of DMSO-cocoa powder extract, in the appropriate dilution, was mixed with 210  $\mu\text{L}$  of DMSO and stirred for 1 min with an orbital shaker. Then, 25  $\mu\text{L}$  of HAuCl<sub>4</sub> solution ( $2.0 \times 10^{-2} \text{ mol L}^{-1}$ ) and 235  $\mu\text{L}$  of phosphate buffer solution (pH 8.0;  $1.0 \times 10^{-2} \text{ mol L}^{-1}$ ) were added (final volume 500  $\mu\text{L}$ ). The solution was mixed with an orbital shaker for 1 min and the reaction was started heating the reaction mix for 5 min at 45 °C in a water bath. Finally, the reaction was blocked at  $-20 \text{ }^\circ\text{C}$  for 5 min to allow measurements in series. The absorbance due to the AuNPs formation was recorded at 540 nm and evaluated after the blank subtraction (the blank was composed by reaction mix without sample).

## 3. Results and discussion

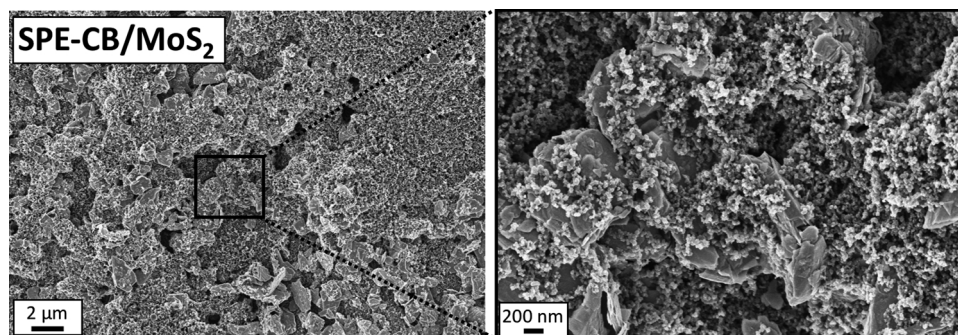
### 3.1. Electrochemical behavior of flavanols on hybrid CB/MoS<sub>2</sub> transducers

In order to study the CB, MoS<sub>2</sub> and the resulting CB/MoS<sub>2</sub> combination, different amounts of the NMs dispersions have been drop casted onto SPE (Section 2.4). Initially, cyclic voltammetry (CV) has been employed to study the electrochemical behavior of the SPE, SPE-CB, SPE-MoS<sub>2</sub> and SPE-CB/MoS<sub>2</sub> electrodes. The attention was focused on the first Catechin (CT) electron transfer reaction that occurs at very low positive potentials because the relevant radical scavenging activity [25,53,62–65]; the CT whole electrochemical behavior is discussed in detail in the supplementary material (Fig. S1). In Fig. 1, the CV curves obtained with 50  $\mu\text{mol L}^{-1}$  of catechin in 0.01  $\text{mol L}^{-1}$  PB (pH 7.0), at  $50 \text{ mV s}^{-1}$  scan rate, have been reported together with the relative blank (PB), performed before and after the catechin scan. In order to confirm the irreversibility of the electrode passivation (or CT attachment), the CT scan was followed by a mild surface regeneration (Section 2.5). Improved electrochemical performances, in term of peak intensity and reversibility, were obtained in all cases compared to bare SPE.

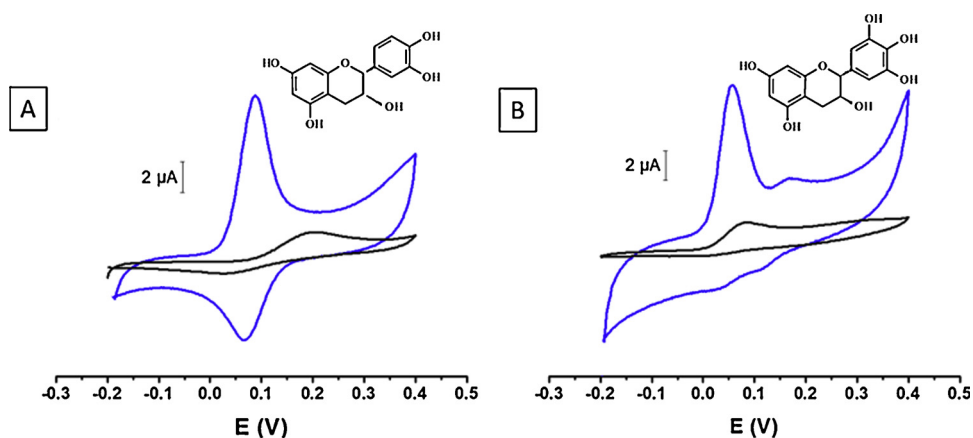
The bare SPE (Fig. 1A) shows the worst response in term of oxidation currents ( $i_{p,a} = 0.84 \mu\text{A}$ ) and peak separation ( $\Delta E_p = 147 \text{ mV}$ ) and an evident 'memory effect' due to analyte's polymerization (blue line) after the scan and the regeneration step (5 CV scan in PB). These results are in accordance with literature [26], and the CV and EIS of Fig. S1 where catechin strongly adsorbed on the electrode surface hindering the electronic transfer. In Fig. 1B is clearly showed that SPE-CB display a significant oxidation current increase ( $i_{p,a} = 4.40 \mu\text{A}$ ) lowering the oxidation potential of CT (SPE-CB  $E_{p,a} = 142 \text{ mV}$  vs. SPE  $E_{p,a} = 198 \text{ mV}$ ) and showing a lower peak-to-peak separation ( $\Delta E_p = 76 \text{ mV}$ ). However, in this case, the CT seems to remain attached to the electrode preserving the electroactivity and showing a residual



**Fig. 1.** Cyclic voltammograms of the bare SPE (A), SPE-CB (B), SPE-MoS<sub>2</sub> (C), and SPE-CB/MoS<sub>2</sub> (D). Black line CV in 0.1 mmol L<sup>-1</sup> PB (pH 7) + 0.1 mol L<sup>-1</sup> KCl, before catechin CV. Red line CV of 50 μmol L<sup>-1</sup> catechins (prepared in 0.1 mmol L<sup>-1</sup> PB + 0.1 mol L<sup>-1</sup> KCl, pH 7). Blue line CV in 0.1 mmol L<sup>-1</sup> PB, pH 7 + 0.1 mol L<sup>-1</sup> KCl, after catechins CV. CVs performed at a scan rate of 50 mV s<sup>-1</sup>. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



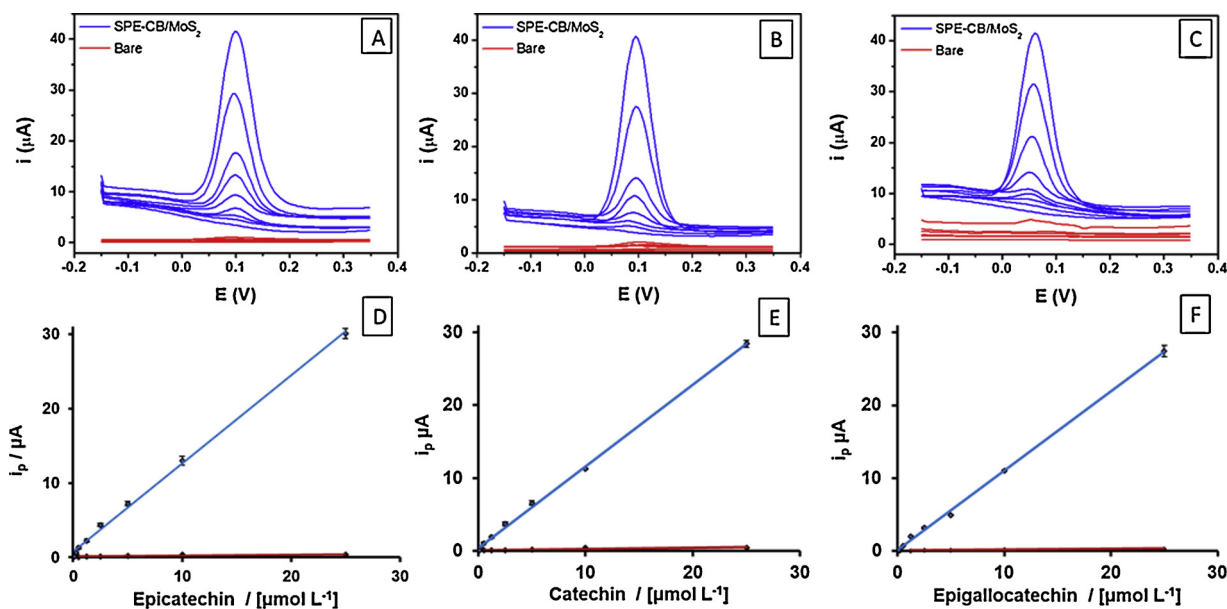
**Fig. 2.** SEM micrograph and magnification of the SPE-CB/MoS<sub>2</sub>.



**Fig. 3.** Cyclic voltammograms of the bare SPE (black line) and SPE-CB/MoS<sub>2</sub> (blue line) performed with 50 μmol L<sup>-1</sup> epicatechin (A) and epigallocatechin (B) prepared in 0.1 mmol L<sup>-1</sup> PB + 0.1 mol L<sup>-1</sup> KCl (pH 7) at a scan rate of 50 mV s<sup>-1</sup>. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

current of the 97% (blue line). Similar behavior has been already reported for NMs, particularly with NMs with oxidized moieties [24] as in the case of CB [36,66]. Indeed, it is reported that carboxyl and/or other oxygen-containing groups can behave as nucleophiles against the o-quinone ring (as Michael acceptor), leading to bond formation between

flavanol and surface active functional groups [24]. On the other hand, the SPE-MoS<sub>2</sub> (Fig. 1C) displays just a slight increase of both oxidation peak ( $i_{p,a} = 0.97 \mu\text{A}$ ) and reversibility ( $\Delta E_p = 88 \text{ mV}$ ) compared with the bare SPE, this because the MoS<sub>2</sub> has a low intrinsic conductivity [44,46,57]. However, it is noteworthy the total absence of residual signal



**Fig. 4.** (A–C) DPV curves obtained using bare SPE (red line) and CB-SPE/MoS<sub>2</sub> (blue line) in presence of increasing concentrations of epicatechin (A), catechin (B) and epigallocatechin (C). (D–F) DPV calibration curves (mean value of three repetitions) obtained using bare SPE (red line) and CB-SPE/MoS<sub>2</sub> (blue line) in presence of increasing concentrations of epicatechin (D), catechin (E) and epigallocatechin (F). For each analyte, the calibration was performed in triplicate, the resulting linear equation (obtained with the mean values,  $n = 3$ ) and determination coefficient obtained for the SPE-CB/MoS<sub>2</sub> are reported in Table 1; while for the bare SPE: (CT)  $y = 0.0168x + 0.0798$ ,  $R^2 = 0.7382$ ; (EP)  $y = 0.0129x + 0.1256$ ,  $R^2 = 0.7316$ ; (EG)  $y = 0.0107x + 0.048$ ,  $R^2 = 0.9406$ . (A–F) DPV conditions: pulse width 50 ms, pulse amplitude 20 mV. The standards were prepared in phosphate-buffered 0.01 mmol L<sup>-1</sup> PB + 0.1 mol L<sup>-1</sup> KCl (pH 7). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**

Analytical characteristics of the SPE-CB/MoS<sub>2</sub> sensor employed for CT, EP, and EG detection.

Analyte	Equation ( $x = \mu\text{mol L}^{-1}$ ; $y = \mu\text{A}$ )	Linear Range ( $\mu\text{mol L}^{-1}$ )	R <sup>2</sup>	Sensitivity ( $\mu\text{A } \mu\text{mol}^{-1}$ )	LOD <sup>a</sup> ( $\mu\text{mol L}^{-1}$ )
CT	$y = 1.122x + 0.413$	0.1–25	0.999	8.93	0.18
EP	$y = 1.185x + 0.762$	0.1–25	0.998	9.43	0.17
EG	$y = 1.092x + 0.090$	0.1–25	0.999	8.69	0.18

<sup>a</sup> LODs were calculated as  $3\sigma/\text{slope}$  ratio, where  $\sigma$  is the standard deviation of the mean value for 10 voltammograms of the blank. Analytical characteristics calculated using the mean value of three calibration curves.

**Table 2**

Analytical characteristics and application of electrochemical sensors employed for catechins detection.

Electrode / Detection Mechanism	Food Matrix (number of samples)	Sample Pretreatment	Comparison methods	Linear Range ( $\mu\text{mol L}^{-1}$ )	LOD ( $\mu\text{mol L}^{-1}$ )	Ref.
GCE/CV	Cocoa powder, chocolate (n = 19)	Defatting/Liquid solid extraction	F.C., total flavonoid, DPPH, ABTS, FRAP	–	–	[38]
Pt-PEDOT/DPV	cocoa-based biscuit (n = 3)	Stirring/filtration on Buchner	–	0.7–8.6 <sup>a</sup>	0.31 <sup>a</sup>	[35]
GR-thermally reduced/DPV	Beer (n = 3)	Dilution	–	1.2–12.0 <sup>b</sup>	–	[33]
GCE-N-doped graphene/DPV	Chinese green tea (n = 4)	Dilution	–	1.0–30 <sup>b</sup>	0.10 <sup>b</sup>	[32]
SPE-CB/MoS <sub>2</sub> /DPV	Cocoa powder (n = 59)	DMSO solubilization	F.C., ABTS, AuNPs	0.1–25.0 <sup>b</sup>	0.17 <sup>b</sup>	[this work]

<sup>a</sup> Epicatechin equivalents.

<sup>b</sup> Catechin equivalents.

(blue line) after the CT scan, this MoS<sub>2</sub> feature, at the best of our knowledge, has not been explored yet and could open new gates to MoS<sub>2</sub> application as an antifouling functional material. Fig. 1D reports the SPE-CB/MoS<sub>2</sub> ability to merge the behavior of both materials. In fact, a significant enhancement of the analytical performance, compared to both the bare (SPE) and the single-NMs modified electrodes (SPE-CB and SPE-MoS<sub>2</sub>) is showed. The ratio CB-MoS<sub>2</sub> 75:25 (v/v) (from now this electrode will be named SPE-CB/MoS<sub>2</sub>) was chosen, because higher amounts of MoS<sub>2</sub> (50 and 75%, v/v) exhibited lower electrochemical improvements, probably due to MoS<sub>2</sub> intrinsic low conductivity [44,46,57]. The SPE-CB/MoS<sub>2</sub> displays a significant

increase in the oxidation current ( $i_{p,a} = 9.7 \mu\text{A}$ ) that occurs at lower potentials ( $E_{p,a} = 98 \text{ mV}$ ). Moreover, a significant decrease in the  $\Delta E_p$  (22 mV) was observed. Considering the obtained  $\Delta E_p$  (near to 30 mV) the reaction involves two electrons that can be attributed to the ‘catecholic ring’ [25]. The pH effect was also studied in Fig. S2 performing CV in a solution containing  $25 \mu\text{mol L}^{-1}$  of CT. The peak intensities obtained at different pH catechin gave a straight line in the 4–8 pH range with a slope of  $57 \text{ mV pH}^{-1}$  unit. Considering these data, we can confirm a fast electron transfer with the same number of protons involved in the electrode reaction. The SPE-CB/MoS<sub>2</sub> modification performed with the 75% of CB and the 25% of MoS<sub>2</sub> was able to totally

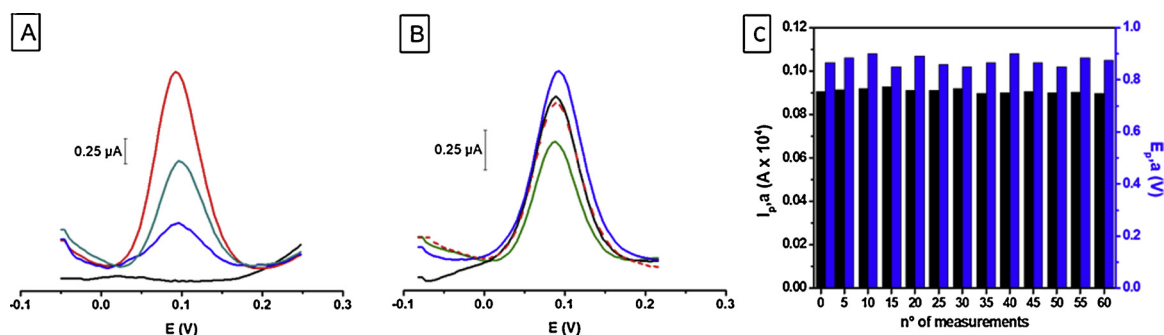


Fig. 5. (A) DPV obtained analyzing a mix of cocoa samples (blue line), fortified with  $0.25 \mu\text{mol L}^{-1}$  (green line) and  $0.5 \mu\text{mol L}^{-1}$  (red line) of epicatechin. (B) DPV of three different samples with low (sample 12, green line), medium (sample 5, black line) and high (sample 1, blue line) polyphenols content. The dashed red line represents the sample 5 measurement repetition, performed after the measurement of the whole set of samples ( $n = 59$ ). (C) DPV Oxidation currents and oxidation potentials obtained with  $10 \mu\text{M}$  epicatechin, used to monitor the SPE-CB/MoS<sub>2</sub> response, during the samples analyzed. Each 5 samples analyzed the measurement has been performed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

prevent both the CT irreversible polymerization and the attachment onto the electrode surface. This behavior was confirmed in Fig. S3, where the EIS of the SPE-CB/MoS<sub>2</sub> does not show any significant differences in the Nyquist plot after 4 consecutive scans of CT, confirming that the charge-transfer ability of the hybrid nanomaterial is not affected. In addition to the excellent electrochemical performance, a remarkable inter-electrode reproducibility was obtained in terms of  $i_{p,a}$  and  $E_{p,a}$  for the CT oxidation peak (RSD 1.2% and 3.5%, respectively,  $n = 10$ ). Figs. 2 and S4 shows the SEM micrographs of the investigated electrodes. The SPE-MoS<sub>2</sub> (Fig. S4) is characterized by MoS<sub>2</sub> 'crystals' with a broad size distribution centred around  $870 \pm 550 \text{ nm}$  (see particle size distribution in Fig. S5), composed by layers of stacked MoS<sub>2</sub> flakes, with the nanometric uniform side (see Fig. S4 SPE-MoS<sub>2</sub> magnification). In the micrograph of the SPE-CB (Fig. S4) it is clearly visible the presence of CB primary units with a mean diameter of  $20 \pm 8 \text{ nm}$  (the particle size distribution is reported in Fig. S5) [67,68]. The SPE-CB/MoS<sub>2</sub> (Fig. 2) shows the co-localization of MoS<sub>2</sub> 'crystals' and carbon black primary particles; the two components interact but maintain their original morphology. The CB intercalation seems to reduce the MoS<sub>2</sub> natural tendency to restacking, with respect to the SPE-MoS<sub>2</sub>, resulting in CB decorated MoS<sub>2</sub> flakes. In particular, the  $\sim 48\%$  of the electrode surface is composed of MoS<sub>2</sub> 'crystals' despite CB is added in a higher amount during the preparation of the electrode. This behaviour can be ascribed to the flakes-like conformation of MoS<sub>2</sub> that favours their floating to the surface. The homogeneity of the obtained surfaces at the nanoscale was further evaluated by measuring the lacunarity of SEM micrographs (see Fig. S6). As expected, the SPE-CB electrode results in more homogeneous surface at the nanometric level, while the lacunarity associated to the SPE-MoS<sub>2</sub> system is higher, in accordance with the broad size distribution of the MoS<sub>2</sub> crystals. Hence, as expected, the SPE-CB/MoS<sub>2</sub> presents an intermediate value of lacunarity with respect to the other two electrodes (SPE-CB and SPE-MoS<sub>2</sub>), according to the co-presence of both CB and MoS<sub>2</sub> morphologies.

In order to assess the exploitability of the SPE-CB/MoS<sub>2</sub> for cocoa polyphenols analysis the proposed electrode has been tested against epicatechin (EP) and epigallocatechin (EG) (Fig. 3). These flavan-3-ols compounds, together with catechin, are the most abundant polyphenolic compounds of cocoa and chocolate and represent the monomers of the proanthocyanidins [3,69].

The SPE-CB/MoS<sub>2</sub> again showed good electrochemical performance despite the EP and EG different chemical structure (see Fig. 3 insets), resulting in different electrochemical behavior. In Fig. 3A it is clearly noticed the EP increased oxidation current ( $i_{p,a} = 10 \mu\text{A}$ ) and the negative shift ( $E_{p,a} = 88 \text{ mV}$ ) in the oxidation potential with respect to the bare SPE ( $i_{p,a} = 1.1 \mu\text{A}$ ,  $E_{p,a} = 198 \text{ mV}$ ). Moreover, in the case of EP (Fig. 3A), increased reversibility in terms of both peak intensity ratio

and peak-to-peak separation was even recorded ( $\Delta E_p = 19 \text{ mV}$ ). The CVs of EG (Fig. 3B), despite the expected irreversible behaviour [70], allowed, also in this case, an  $E_{p,a}$  negative shift (SPE-CB/MoS<sub>2</sub>  $E_{p,a} = 69 \text{ mV}$  vs SPE  $E_{p,a} = 120 \text{ mV}$ ) and a significantly improved anodic peak intensity ( $i_{p,a} = 9.1 \mu\text{A}$ ) compared to the bare SPE ( $i_{p,a} = 1.6 \mu\text{A}$ ). Even with EP and EG, the SPE-CB/MoS<sub>2</sub> totally prevent both the flavanols irreversible polymerization and the attachment to the electrode surface. In addition, despite the different structures of CT, EP and EG a very close oxidation current and oxidation potential have been obtained for all the compounds.

### 3.2. Analytical performance of hybrid CB/MoS<sub>2</sub> transducers for catechins determination

The analytical performance of the SPE-CB/MoS<sub>2</sub> sensor for quantitative analysis was carefully evaluated by studying the response toward CT, EP and EG using differential pulse voltammetry (DPV) under optimized parameters (see Section 2.5). Fig. 4 shows the voltammograms and the calibration curves obtained for the assayed concentrations of EP (A and D), CT (B and E) and EG (C and F) using the SPE-CB/MoS<sub>2</sub>. Analytical figures of merit for the SPE-CB/MoS<sub>2</sub> towards CT, EP and EG detection are listed in Table 1.

Satisfactory linear range, sensitivity, and limit of detection were achieved in all the cases. Interestingly, all these analytical parameters are similar among them confirming the same anodic reactivity for the three analytes allowing the quantification of the flavanols index content with reliability. In addition, intra-electrode repeatability has been further confirmed by the RSD obtained with twenty different measurements performed with a solution containing  $10 \mu\text{mol L}^{-1}$  epicatechin:  $i_{p,a} < 1.1\%$  and  $E_{p,a} < 3.2$ . The 'poor linearity' (Fig. 4 red curves) obtained for the bare SPE ( $R^2 = 0.9406 - 0.7316$ ) is in accordance with the  $E_{p,a}$  positive shift ( $E_{p,a}$ , average shift of  $200 \text{ mV}$ ), that occurs for all the analytes, indicating an increase of the electrode surface passivation. Moreover, the lower sensitivity (about 2 orders of magnitude vs SPE-CB/MoS<sub>2</sub>) of the bare SPE towards all the analytes is evident (Fig. 4).

Table 2 lists the performance of the CB-SPE/MoS<sub>2</sub> compared with other sensors and nanomaterial-based sensors for catechin detection.

In this work, the obtained LODs and linear ranges are comparable or better than those reported in the literature, even when applied to other food matrices. It should be emphasized that among the reported works for the analysis of catechin, this is the only case using screen-printed technology. Importantly, no one of the reported works pays attention to the fouling process, that is the principal drawback for the catechin detection. In this study, the attention has been paid not only to the proposed sensor performances but even to the success and the exploitability of the application in complex samples, using a significant number of samples and comparing the results with other well-

**Table 3**  
Analysis of cocoa samples by the SPE-CB/MoS<sub>2</sub> and the F.C, ABTS and AuNPs assays<sup>a</sup>.

Sample	SPE-CB/MoS <sub>2</sub> (mg g <sup>-1</sup> )	Folin-Ciocalteu (mg g <sup>-1</sup> )	ABTS (mg g <sup>-1</sup> )	AuNPs (mg g <sup>-1</sup> )
1	86.1 ± 0.3	86.0 ± 0.5	72.5 ± 0.3	86.0 ± 4.8
2	50.4 ± 0.6	45.3 ± 0.5	40.2 ± 0.3	49.2 ± 0.3
3	34.1 ± 0.3	31.2 ± 1.6	28.2 ± 1.2	37.2 ± 0.2
4	59.9 ± 0.5	48.7 ± 0.2	37.6 ± 2.4	47.5 ± 0.1
5	39.2 ± 0.5	32.2 ± 2.2	22.6 ± 0.6	29.7 ± 0.6
6	55.1 ± 1.3	49.2 ± 2.3	42.1 ± 1.1	50.1 ± 4.4
7	37.9 ± 0.4	27.7 ± 2.1	21.7 ± 0.2	30.9 ± 3.7
8	58.4 ± 1.4	52.3 ± 2.3	39.3 ± 1.8	52.2 ± 4.3
9	36.7 ± 1.1	30.1 ± 1.1	25.0 ± 1.1	32.0 ± 1.8
10	26.6 ± 1.1	17.4 ± 0.7	186 ± 1.0	26.3 ± 1.8
11	31.9 ± 0.5	19.2 ± 0.7	21.2 ± 0.3	23.9 ± 1.3
12	26.0 ± 1.0	16.0 ± 0.6	18.7 ± 0.4	20.2 ± 0.6
13	14.5 ± 0.4	3.7 ± 0.1	4.1 ± 0.1	11.5 ± 0.1
14	14.4 ± 0.5	3.4 ± 0.4	3.6 ± 0.5	11.5 ± 0.3
15	23.2 ± 0.6	16.2 ± 1.1	17.9 ± 0.3	20.3 ± 0.9
16	28.0 ± 0.4	20.0 ± 1.9	20.3 ± 0.3	25.1 ± 1.0
17	21.9 ± 1.0	8.8 ± 0.2	9.3 ± 0.1	16.7 ± 1.2
18	25.0 ± 0.6	12.5 ± 0.5	9.4 ± 0.9	19.9 ± 0.9
19	15.7 ± 0.3	3.4 ± 0.1	3.1 ± 0.5	11.0 ± 0.6
20	22.2 ± 0.6	11.1 ± 0.1	10.8 ± 0.9	18.3 ± 0.4
21	15.0 ± 0.6	4.4 ± 0.1	3.2 ± 0.1	11.5 ± 0.4
22	14.6 ± 0.5	2.5 ± 0.1	2.7 ± 0.5	10.0 ± 0.2
23	14.5 ± 0.3	2.7 ± 0.1	3.0 ± 0.1	10.6 ± 1.2
24	14.9 ± 0.5	3.4 ± 0.1	3.0 ± 0.0	10.5 ± 0.6
25	29.0 ± 0.9	20.7 ± 0.7	21.4 ± 2.0	22.3 ± 3.1
26	42.0 ± 1.1	29.9 ± 0.9	34.4 ± 0.6	42.4 ± 1.3
27	27.3 ± 0.7	24.2 ± 0.1	21.4 ± 2.0	24.7 ± 0.8
28	43.0 ± 1.5	27.6 ± 1.0	23.6 ± 0.4	30.3 ± 1.8
29	31.6 ± 0.8	20.1 ± 0.1	18.5 ± 0.8	24.0 ± 0.3
30	15.0 ± 0.5	5.2 ± 0.2	4.3 ± 0.0	11.6 ± 0.2
31	16.1 ± 0.4	5.6 ± 0.3	4.9 ± 0.6	13.1 ± 0.7
32	36.3 ± 0.5	27.4 ± 1.2	20.9 ± 0.0	27.4 ± 0.1
33	41.6 ± 0.1	25.8 ± 0.3	22.8 ± 0.1	28.5 ± 0.9
34	21.5 ± 0.1	4.6 ± 0.5	3.7 ± 0.5	10.5 ± 0.5
35	31.2 ± 0.3	13.2 ± 1.0	15.3 ± 0.8	23.1 ± 1.7
36	44.4 ± 1.1	27.3 ± 1.9	23.2 ± 1.0	30.7 ± 1.1
37	31.3 ± 0.8	22.7 ± 0.9	16.3 ± 0.1	25.7 ± 0.4
38	35.5 ± 1.1	27.2 ± 0.2	15.0 ± 0.5	30.8 ± 0.3
39	31.9 ± 0.4	17.3 ± 0.7	23.3 ± 0.1	32.4 ± 4.2
40	22.4 ± 0.4	3.8 ± 0.1	3.4 ± 0.5	12.6 ± 0.6
41	21.8 ± 0.1	7.8 ± 0.2	5.0 ± 0.1	12.4 ± 0.3
42	15.1 ± 0.2	3.6 ± 0.5	3.3 ± 0.0	10.8 ± 0.8
43	22.7 ± 0.5	5.7 ± 0.2	4.3 ± 0.1	13.4 ± 1.5
44	13.1 ± 0.4	3.5 ± 0.0	3.4 ± 0.5	10.6 ± 0.3
45	31.0 ± 0.1	29.1 ± 0.1	29.7 ± 0.9	34.0 ± 2.0
46	21.7 ± 0.3	10.7 ± 0.7	10.5 ± 0.2	15.6 ± 0.8
47	21.7 ± 0.7	10.1 ± 0.4	13.8 ± 0.1	18.5 ± 1.9
48	22.3 ± 0.2	11.6 ± 0.6	12.8 ± 0.9	18.5 ± 0.4
49	55.2 ± 1.4	47.2 ± 3.9	38.6 ± 1.9	45.8 ± 0.3
50	22.5 ± 0.8	10.6 ± 0.6	10.0 ± 0.5	16.6 ± 0.5
51	32.6 ± 1.0	18.3 ± 2.0	27.3 ± 0.7	25.8 ± 0.2
52	14.7 ± 0.2	2.6 ± 0.2	2.6 ± 0.5	9.9 ± 0.4
53	19.7 ± 0.5	5.9 ± 0.2	3.2 ± 0.5	12.7 ± 1.0
54	31.6 ± 1.0	29.9 ± 0.1	28.1 ± 0.8	28.9 ± 0.2
55	59.0 ± 0.4	42.5 ± 0.1	38.2 ± 0.6	44.6 ± 0.3
56	34.2 ± 0.5	28.5 ± 0.3	24.6 ± 2.1	32.9 ± 1.9
57	49.2 ± 1.4	37.8 ± 0.1	30.9 ± 0.5	42.2 ± 1.2
58	54.5 ± 1.4	49.2 ± 7.1	41.4 ± 0.4	54.5 ± 0.8
59	43.4 ± 0.5	35.6 ± 2.6	30.0 ± 0.5	35.6 ± 1.6

<sup>a</sup> The data are expressed as epicatechin equivalents as mean values ± SD, n = 3. Calibration curves (performed with epicatechin; x = μmol L<sup>-1</sup> and y = Abs.) showed both good linearity and coefficients of determination: F.C. y = 0.1587x - 0.0032, R<sup>2</sup> = 0.9971; ABTS y = 69.682x + 0.7792, R<sup>2</sup> = 0.9925; AuNPs y = 0.0291x - 0.0753, R<sup>2</sup> = 0.9940.

established methods (Section 3.3).

Recovery (Fig. 5A) and renewability/regenerability (Fig. 5B) studies were also evaluated using a representative mix of cocoa powder samples with different polyphenol content (accordingly Section 2.3).

The voltammograms obtained for the mix of samples (Fig. 5A, blue

line), and the fortified samples (Fig. 5A, green and red lines), exhibited identical E<sub>p,a</sub> (90 ± 5 mV) and excellent RSD for i<sub>p,a</sub> ≤ 1.2%, (n = 3) as well as quantitative recoveries with values ranging from 94% to 103% demonstrating the sensor exploitability for the catechins determination in complex samples.

Fig. 5B shows the voltammograms obtained with cocoa samples with low (sample 12, green line), medium (sample 5, black line) and high (sample 1, blue line) polyphenols content (each diluted at the adequate dilution to fit the signal in the calibration plot). Noteworthy is the very high regenerability showed by the SPE-CB/MoS<sub>2</sub>. This behavior was proven by monitoring the electrode state during the 59 sample measurements (interspersed with a fast surface regeneration see Section 2.5). The SPE-CB/MoS<sub>2</sub> regenerability is further underlined in Fig. 5B, where the dashed red line represents the Sample 5 measured after measuring the whole set of samples, obtaining a signal recovery of 99%. These results are in accordance with previously discussed results (see Section 3.1) and confirm the ability of the SPE-CB/MoS<sub>2</sub> to avoid the permanent catechins polymerization or attachment onto the electrode surface.

To prove the sensor surface regenerability, the whole set of samples (59 cocoa powders) has been measured on the same SPE-CB/MoS<sub>2</sub>, and the electrode 'state' has been monitored measuring epicatechin standard (10 μmol L<sup>-1</sup>) every 5 samples measured (Fig. 5C). Impressively, Fig. 5C, shows a very stable response obtained that result in RSD of the signal obtained of 0.9% and 5.2% for the I<sub>p,a</sub> and E<sub>p,a</sub>, respectively.

### 3.3. Quantitative sample analysis of total catechins on hybrid CB/MoS<sub>2</sub> transducers

Then the applicability of the CB-SPE/MoS<sub>2</sub> for the determination of flavanols was rigorously carried out through the analysis of a wide set of cocoa samples (n = 59). Table 3 lists the quantitative analysis of cocoa samples by the proposed SPE-CB/MoS<sub>2</sub> sensor. In order to demonstrate analytical reliability of the proposed electrochemical sensor, cocoa powder samples were also analyzed by the classical F.C. (total polyphenols) and ABTS (antioxidant capacity) assays as well as by AuNPs-based free-extraction approach [59]. Particular attention was paid on the AuNPs method, which relies on the direct formation of AuNPs in organic solvent (DMSO)-aqueous medium, driven by endogenous polyphenols. This 'extraction-free' approach involves the use of DMSO acting as an organic solvent, able to 'interact', at the same time, with the fatty matrix and with the aqueous medium of measurement, making the cocoa polyphenols analysis faster and easier. The effectiveness of this DMSO-based approach was tested with the set of 59 cocoa samples and compared both with a conventional liquid-liquid extraction (LL) methods which need a defatting step [59] (Section 2.3) and with the well-established methods of Folin-Ciocalteu and ABTS. The effectiveness of the proposed DMSO-based approach was confirmed by the good correlations (Fig. S7) obtained between DMSO-based and the LL conventional method (F-C: r = 0.954 and ABTS: r = 0.971, n = 59) confirming the suitability of the DMSO-based extraction-free approach. Furthermore, the DMSO-based approach requires a lower amount of sample (0.1 g vs. 8.0 g), a significant lower solvent consumption occurs (1.5 ml of DMSO vs. 150 ml of hexane and 5 ml of 70:29.5:0.5 acetone/water/acetic acid) as well as a reduction in waste generation; moreover, the procedure is not time-consuming (15' min vs. 8 h). Therefore, the DMSO-based free-extraction approach was selected for the quantitative determination of polyphenols in cocoa samples using the proposed SPE-CB/MoS<sub>2</sub> electrochemical sensor (see Table 3).

Despite heterogeneity of the samples, in terms of cocoa types, fat content (5.6–26.1%), color and polyphenols content, the data obtained with the SPE-CB/MoS<sub>2</sub> resulted highly correlated with all the well-established methods assays FC (r = 0.972) and ABTS (r = 0.966) as well as with the AuNPS-based (r = 0.949).

#### 4. Conclusions

Herein an electrochemical sensor based on the synergic employment of MoS<sub>2</sub> and CB is successfully applied to catechins determination in complex cocoa samples. The SPE-CB/MoS<sub>2</sub> electrodes merge the CB ability to enhance the electrochemical response and the MoS<sub>2</sub> 'anti-fouling' properties against catechins, drawbacks that occur to both conventional carbons and NMs-modified electrodes. To the best of our knowledge, this latter MoS<sub>2</sub> hidden feature has been deeply studied and proven for the first time in this article. The realized sensor showed an impressive stable and reproducible response, with low working potentials, excellent resistance to fouling with detection limits in the nanomolar range. Noteworthy, the whole set of 59 samples have been measured using a single SPE-CB/MoS<sub>2</sub>, without loss of signal, proving it excellent 'antifouling' properties. Moreover, in order to speed up and simplify, the cocoa powder polyphenols analysis (that required 8 h), a fast (15 min) direct cocoa polyphenols DMSO-based solubilization extraction-free strategy is proposed. Definitely, the proposed approach is clearly intended for fast (total time 15' min, from sampling to analysis) evaluation of polyphenols in cocoa powder, the fast DMSO 'extraction' (15 min, without volatile solvents) together to the SPE-CB/MoS<sub>2</sub> (re-generable and portable) allows a real delocalization of the analysis and could be extended for the catechins analysis in other food matrices. The 'DMSO-based polyphenols approach results rapid, effective and eco-friendly (total working volume 1.5 mL), does not require evaporation steps (and volatile compounds) and can be coupled with cocoa polyphenols (and antioxidant capacity) content evaluation methods. Definitely, the SPE-CB/MoS<sub>2</sub> showed an important synergistic effect and the combination of these nanomaterials can offer new opportunities to develop new devices and applications. In our case the proposed SPE-CB/MoS<sub>2</sub> overcome the proof of applicability, becoming an analytical tool able to overtake the classics catechins analytical drawbacks.

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#### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.snb.2019.126651>.

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