



# Carbon based electrodes for the voltammetric determination of capsaicin in spicy samples

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## ABSTRACT

A voltammetric sensor based on the modification of a screen-printed carbon electrode with reduced graphene oxide (rGO-SPCE) is presented for the cyclic voltammetric determination of capsaicin in a wide range of concentrations. Special attention was given to factors affecting sensitivity and repeatability in order to overcome common reported issues related to the voltammetric determination of capsaicin. Fouling associated to the adsorption of capsaicin was minimized using a simple and effective washing step, allowing sensors to be reused for at least 10 measurements with a repeatable signal. The developed rGO-SPCE presented two well-defined linear ranges (from 1.1 to 25.0 and from 25.0 to 150.3  $\mu\text{mol L}^{-1}$ ) and provided an increased sensitivity as compared to the unmodified electrode as well as to other modified electrodes based on carbon nanomaterials commercially available. Furthermore, a low limit of detection of 0.3  $\mu\text{mol L}^{-1}$  was achieved and the accuracy of the method was demonstrated for the determination of capsaicin in chilli pepper, paprika samples and Tabasco sauce, obtaining comparable results to those achieved by liquid chromatography with UV–vis detection.

## 1. Introduction

Hot peppers are an important ingredient in several worldwide cuisines, being used as either vegetable, condiment or spice since they provide valuable sensorial attributes including colour, spiciness, and flavour. Capsaicin (8-methyl-N-vanillyl-*trans*-6-nonenamide) is one of the major capsaicinoids present in hot peppers and, together with dihydrocapsaicin, is responsible for approximately 90% of their spiciness [1,2]. As such, the determination of capsaicin is relevant for food quality assessment, not only to assess the pungency level in different pepper varieties but also for the characterization and geographical origin authentication of spices like paprika [3,4]. In addition, capsaicin also serves as a common marker for the identification of waste oil because it remains intact even after cooking and processing procedures [5]. Moreover, outside of the food field, capsaicin is also employed in numerous pharmaceutical products due to its beneficial therapeutic activity providing, among others, analgesic, anti-obesity, anti-inflammatory, anti-cancer, anti-oxidant, and neuro-protective functions [6].

Furthermore, the irritant properties of capsaicin have promoted its use as riot control agents and self-defence sprays [7].

In the food field, pungency levels were traditionally evaluated by means of the Scoville test, an organoleptic test based on a human panel; in this, foods are classified into five levels of pungency based on the number of times that a product needs to be diluted until its pungency can no longer be detected. However, this test is highly subjective and dependent on the fatigue and taste threshold of each panel member [8], which has promoted the development of analytical methods for the determination of capsaicin as a more suitable approach for the assessment of pungency levels. Several analytical techniques have been reported for the determination of capsaicin including high-performance liquid chromatography (HPLC) [1,9], gas chromatography (GC) [10], mass spectrometry (MS) [11], spectroscopy [12] and electrochemical methods [13,14]. The latter offer valuable features such as rapid analyses, economic and miniaturized equipment, and the possibility to perform on-site analyses while maintaining a high sensitivity and low limits of detection.

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The electrochemical detection of capsaicin has been reported by different types of electrodes including carbon nanomaterials [15,16], metal oxides [14,17], metal–organic frameworks [18], and molecularly imprinted polymers [19,20]. Among them, carbon nanomaterials present many attractive features such as large surface to volume ratio, high conductivity, high physical and chemical stability, and an organic and biocompatible nature [21]. In particular, reduced graphene oxide (rGO) is a cheaper and easier-to-fabricate alternative to graphene that has extensively been reported for electroanalytical purposes [22]. Nevertheless, electrochemical determination of capsaicin using electrodes based on carbon or carbon nanomaterials is often hindered by fouling effects [14,16,23], an issue that has promoted research on the prevention of fouling and /or the minimization of its effects in accuracy and reproducibility. Thus, in this work we report a rGO modified screen-printed carbon electrode (rGO-SPCE) for the determination of capsaicin by cyclic voltammetry (CV) at the  $\mu\text{mol L}^{-1}$  level in food samples. In addition, a fast and effective chemical cleaning is proposed to minimize the effects of fouling, significantly improving measurement repeatability and allowing accurate results in the determination of capsaicin in several food products, providing comparable values to those obtained by HPLC with UV–vis detection (HPLC-UV).

## 2. Experimental section

### 2.1. Chemicals

Capsaicin, perchloric acid, hydrochloric acid, disodium hydrogen phosphate, and graphene oxide were supplied by Sigma-Aldrich (St. Louis, MO, USA). Potassium chloride, potassium ferrocyanide, and potassium hydroxide were acquired from Merck (Darmstadt, Germany). Acetonitrile and methanol, both HPLC gradient grade, were purchased from Fisher Scientific (Hampton, NH, USA). Potassium ferricyanide and formic acid 98% were supplied from Panreac (Barcelona, Spain). Acetone and sodium phosphate monobasic were provided by Acros Organics (Geel, Belgium) and Scharlab (Barcelona, Spain), respectively.

Capsaicin stock solutions were prepared weekly in acetone and stored in the fridge. All other capsaicin solutions were prepared daily by appropriate dilution in acetone. Unless otherwise indicated, the remaining solutions were prepared in ultrapure water (Milli-Q® Reference A<sup>+</sup> System, Merck Millipore, Millipore).

### 2.2. Instrumentation

The voltammetric determination of capsaicin was performed by means of a PGSTAT 12 multipotentiostat (Ecochemie, The Netherlands) connected to a 663 VA stand (Metrohm Switzerland) and controlled by GPES software (version 4.9.007, Ecochemie). Voltammetric measurements were carried out in a 3 electrode configuration cell using the working electrode from a screen-printed electrode (either commercial or modified as explained in section 2.2) and external Ag/AgCl/KCl (3 M) reference and Pt counter electrodes, both supplied by Metrohm (Switzerland). For comparison purposes, the following commercial screen-printed electrodes were acquired from Metrohm-Dropsens (Oviedo, Spain): carbon (ref. DRP-110), carbon nanotubes (ref. DRP-110 CNT), carbon nanofibers (ref. DRP-110 CNF), and graphene (ref. DRP-110 GPH). Working electrodes were connected to the potentiostat by means of a flexible cable (ref. CAC, Metrohm-Dropsens, Oviedo, Spain). All potentials are referred to the Ag/AgCl/KCl (3 M) reference electrode.

### 2.3. Preparation of rGO-SPCE

rGO-SPCE was prepared by modification of a 4 mm diameter commercial carbon screen-printed electrode (ref. DRP-110, Metrohm-DropSens, Oviedo, Spain) with graphene oxide by drop-casting and further electrochemical reduction. For this purpose, a  $2 \text{ mg L}^{-1}$  of

graphene oxide dispersion was prepared in milliQ water and stored in the refrigerator until further use. This solution was sonicated for 15 min prior to its use to ensure a homogeneous dispersion. Then, 10  $\mu\text{L}$  of this dispersion were drop-casted onto the working electrode while protecting the reference and counter electrode with a face mask made of polypropylene adhesive film. Solvent was allowed to evaporate for 1 h at 25 °C before the mask was removed, thus obtaining a homogeneous layer of graphene oxide.

Electrochemical reduction of graphene oxide was performed by cyclic voltammetry (CV), applying 15 cycles from 0.6 to  $-1.4 \text{ V}$  at  $20 \text{ mV s}^{-1}$  in  $0.1 \text{ mol L}^{-1}$  phosphate buffer (pH 7.4). This resulted in a reduction peak at ca.  $-0.4 \text{ V}$ , which confirmed the reduction of graphene oxide (See Fig. S1, in Supplementary Material).

### 2.4. Voltammetric determination of capsaicin

CV measurements were carried out in  $0.01 \text{ mol L}^{-1}$  HCl containing  $0.1 \text{ mol L}^{-1}$  KCl. Capsaicin was adsorbed onto the working electrode for 300 s under stirring and, subsequently, 3 CV cycles were recorded from  $-0.3$  to  $1.0 \text{ V}$  at  $100 \text{ mV s}^{-1}$ . In between measurements, electrodes were cleaned to avoid fouling effects by immersing the electrode for 3 min in a 20% acetone and 80% perchloric acid  $0.1 \text{ mol L}^{-1}$  solution.

In order to compensate for electrode variability within the same commercial batch, the signal obtained for capsaicin was normalized for each electrode using the signal obtained in ferrocyanide / ferricyanide solution prior to electrode modification. For this purpose, the oxidation current obtained in the third cycle for capsaicin was divided by the oxidation current obtained for ferrocyanide / ferricyanide.

### 2.5. Sample analysis

Two different branches of powdered paprika condiment (La Vera and a white-label brand), one red chilli pepper and one Tabasco™ sauce were purchased from local supermarkets, and their capsaicin content was analysed using the proposed voltammetric method and HPLC-UV as a reference analytical technique (see Supplementary Information). Powdered paprika and Tabasco™ sauce were employed as received whereas chilli pepper was grounded prior to capsaicin extraction. HPLC analysis and extraction procedures are detailed in the Supplementary Information and were based on previously described procedures [4,14].

For voltammetric measurements, 1.4 – 3.5 mL of extract (depending on the considered sample) were added to a voltammetric cell containing 30 mL of  $0.01 \text{ mol L}^{-1}$  HCl with  $0.1 \text{ mol L}^{-1}$  KCl and CV measurements were carried out following the above-mentioned conditions. Samples were analysed in triplicate by means of the standard addition method, performing a total of 4 additions onto the original sample.

### 2.6. Extraction procedure and HPLC-UV analysis

The extraction procedure was carried out in triplicate according to the following procedure: 7 mL of methanol were added to 1 g of sample and the resulting mixture was sonicated for 15 min and magnetically stirred for 2 h. The obtained dispersion was filtered using a glass wool filter paper, the extracted liquid was collected in a 10 mL volumetric flask, and methanol was added until the volume mark.

HPLC measurements were carried out with an Agilent 1200 Series HPLC instrument equipped with a G1322A degasser, a G1311A quaternary pump, and a G1329A autosampler and controlled with the Agilent Chemstation software, all from Agilent Technologies (Palo Alto, CA, USA). The chromatographic method, which employs a Kinetex C18 ( $100 \times 4.6 \text{ mm}$ ,  $100 \text{ \AA}$ ,  $5 \mu\text{m}$ ) column from Phenomenex (Torrance, CA, USA), a mobile phase based on methanol and 0.1 % formic acid with gradient elution, and UV detection at 280 nm, is reported elsewhere. The injection volume was 10  $\mu\text{L}$ .

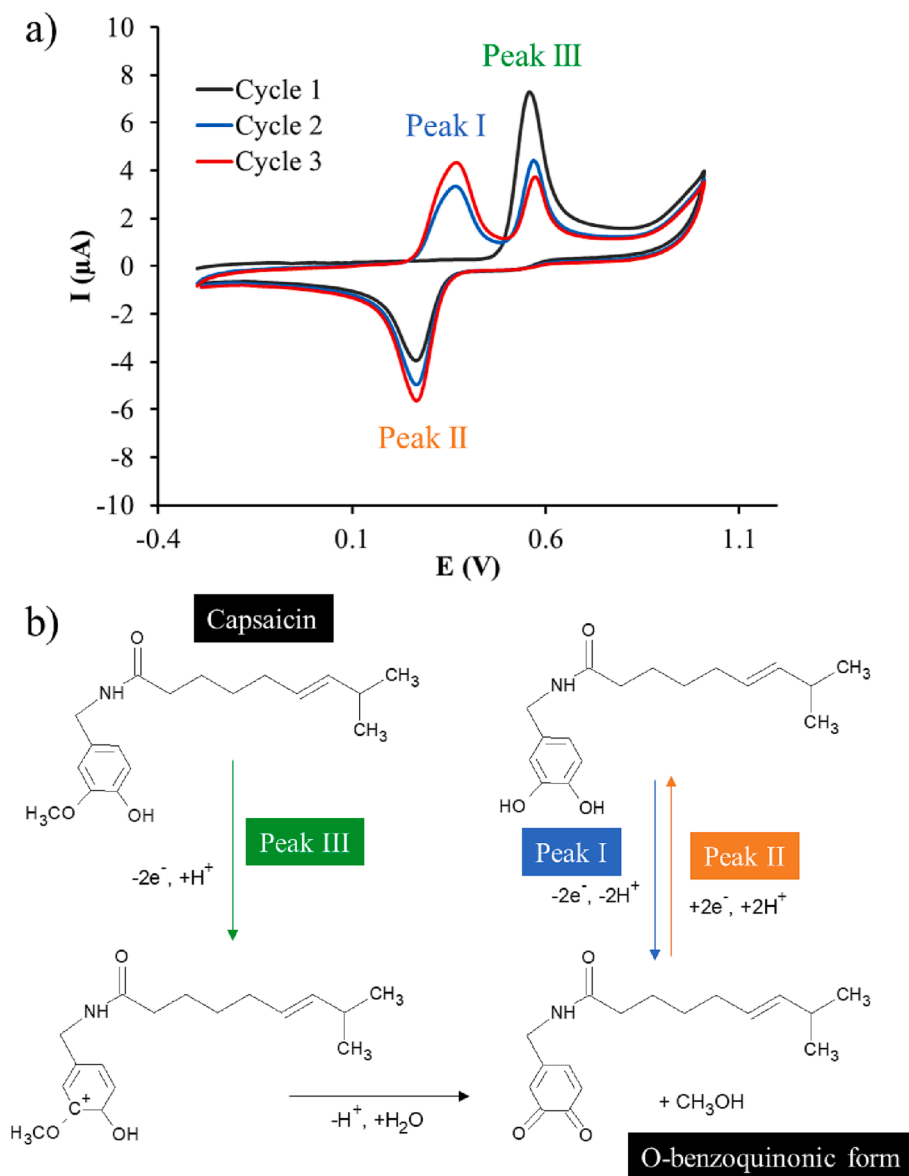
### 3. Results and discussion

#### 3.1. Voltammetric determination of capsaicin on carbon electrodes

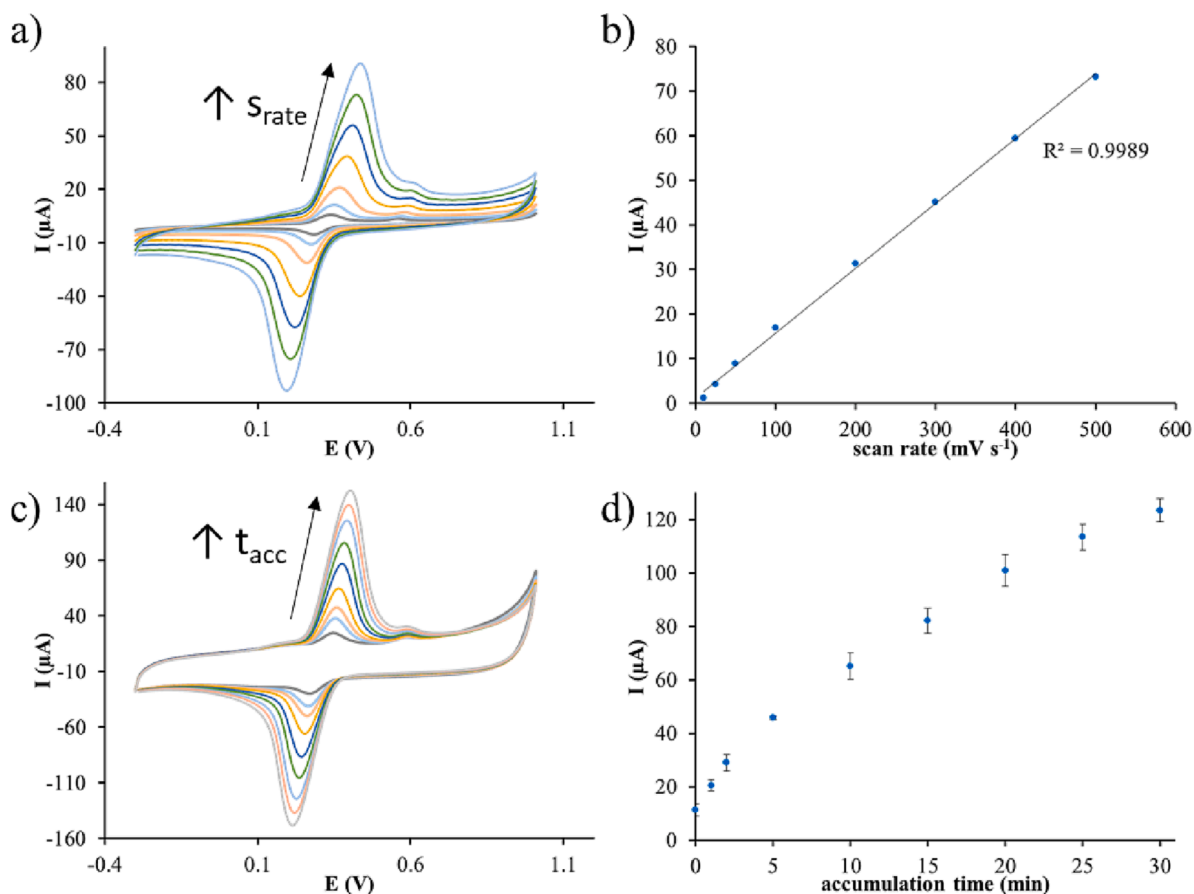
The voltammetric behaviour of capsaicin in carbon electrodes was first studied using an unmodified SPCE in order to understand the oxidation–reduction process. Fig. 1a shows three consecutive cyclic voltammograms for a solution containing  $50 \mu\text{mol L}^{-1}$  capsaicin in  $0.01 \text{ mol L}^{-1}$  HCl with  $0.1 \text{ mol L}^{-1}$  KCl. As it can be observed, the first cycle presents a prominent oxidation peak at ca.  $0.566 \text{ V}$  (peak III) and a reduction peak at ca.  $0.264 \text{ V}$  (peak II). As the number of cycles increases, the intensity of peak III decreases while a second oxidation peak appears (peak I) at ca.  $0.365 \text{ V}$  and the intensity of the reduction peak increases. Peak I corresponds most probably to the oxidation process associated to the reduction in peak II, with a peak-to-peak separation of  $101 \text{ mV}$ . This electrochemical behaviour has been previously reported and attributed to the oxidation mechanism presented in Fig. 1b [24,25], in which the guaiacol ring of capsaicin is first electrochemically oxidized to a highly unstable carbon carbocation that is quickly hydrolyzed in an irreversible chemical process that produces the o-benzoquinonic form of

capsaicin, giving rise to peak III. The obtained o-benzoquinonic form is reversibly reduced to catechol in a process involving 2 electrons, which is observed as peaks II and I. This mechanism justifies the absence of peak I in the first cycle as well as the decrease of peak III and increase of peak I in subsequent cycles. Nevertheless, the signal change significantly decreases after 3 cycles and peak I stabilizes at this point, making the oxidation current of peak I in the third cycle a suitable signal for analytical purposes.

After selecting an appropriate analytical signal, scan rate was optimized considering values from  $10$  to  $500 \text{ mV s}^{-1}$ . As it can be observed in Fig. 2a, increasing the scan rate has a significant effect in peaks I and II while peak III is almost unaffected. As expected, higher scan rates result in increased peak currents. However, it is worth noting that peak-to-peak separation is also shifting with scan rate and peak width is enlarging, promoting the overlapping of peaks I and III. As this overlapping will hinder the sole integration of peak I and decrease the reliability of the method, a scan rate of  $100 \text{ mV s}^{-1}$  was selected as optimal. A closer examination of the variation of peak current with the scan rate (Fig. 2a) demonstrates a linear relationship, highlighting the presence of electrode-adsorbed species. Thus, as the determination of capsaicin will



**Fig. 1.** (a) Consecutive cyclic voltammograms for a solution containing  $50 \mu\text{mol L}^{-1}$  capsaicin in  $0.01 \text{ mol L}^{-1}$  HCl with  $0.1 \text{ mol L}^{-1}$  KCl using an unmodified SPCE and a scan rate of  $100 \text{ mV s}^{-1}$ . (b) Proposed mechanism for capsaicin oxidation process.



**Fig. 2.** Effect of scan rate (a-b) and accumulation time (c-d) in the cyclic voltammetric determination of  $50 \mu mol L^{-1}$  capsaicin in  $0.01 mol L^{-1}$  HCl with  $0.1 mol L^{-1}$  KCl using an unmodified SPCE. Scan rate optimization (a) was performed without accumulation time and the relationship between current and scan rate was evaluated (b). Optimization of accumulation time (c) was carried out at  $100 mV s^{-1}$  and current was represented against accumulation time (d). For error bars  $n = 3$ .

highly be affected by its adsorption on the electrode surface, accumulation time was also optimized considering values from 0 to 30 min. Accumulation of capsaicin was performed under constant stirring at open circuit potential and resulted in a significant increase of the signal with prolonged times (Fig. 2c and 2d). The rate of increase was particularly elevated in the first minutes but decreased overtime. Thus, an accumulation of 5 min was selected as a compromise between increased capsaicin signal and short analysis time.

Although adsorption of capsaicin enhances the electrochemical signal, it also presents a negative side-effect as adsorption derives in fouling effects that hinder the reusability of the electrode [14,16,23]. The repercussions of this issue are observed in Fig. S2a, in which it can be observed that capsaicin peak significantly increases as successive measurements are carried out with the same electrode, with the tenth measurement providing a peak more than three times higher than that obtained in the first measurement. This signal increase was definitely associated to the accumulation of capsaicin on the electrode surface, as it was confirmed by the presence of capsaicin signal when a blank solution was measured right after measuring a solution containing  $50 \mu mol L^{-1}$  capsaicin (Fig. S2b). Thus, removal of capsaicin from the electrode surface in-between measurements was of utmost importance and a desorption procedure was investigated. Electrochemical desorption was not successful and thus, a chemical procedure was attempted. For this purpose, the electrode was immersed into a washing solution for 3 min in-between measurements. Several options were considered as washing solutions including potassium hydroxide, perchloric acid, acetone and binary mixtures of these solutions. Potassium hydroxide  $0.1 mol L^{-1}$  was able to completely eliminate capsaicin from the electrode surface but it also damaged the electrode surface, which resulted

in a progressively increasing capacitive current that prevented capsaicin determination after 5 measurements. Washing with acetone also damaged the SPE whereas perchloric acid did not completely allow the removal of capsaicin. Regarding the use of binary mixtures, the best results were obtained using a mixture of perchloric acid  $0.1 mol L^{-1}$  and acetone in a 4:1 proportion. Although an increase in the capacitive signal was also observed, this was constant after the first washing step and highly repetitive measurements were achieved (Fig. S2c), with no capsaicin signal observed in blanks recorded after the washing step (Fig. S2d). Thus, perchloric acid  $0.1 mol L^{-1}$  acetone (1:4) was selected as optimal washing solution.

Using the above-mentioned optimized conditions, the analytical performance of the SPCE was assessed in terms of sensitivity (slope of the calibration curve), linear range, and limits of detection (LOD) and quantification (LOQ), which were calculated as three and ten times, respectively, the standard deviation of the intercept over the slope of the calibration curve (Table 1). For this purpose, calibration curves were recorded from  $0.1$  to  $150.0 \mu mol L^{-1}$  capsaicin (Fig. 3a). Capsaicin signal increased with concentration resulting in two well-defined linear ranges as displayed in Fig. 3b and the LOD, calculated using the first linear range, was below  $1.5 \mu mol L^{-1}$ . Furthermore, repeatability was calculated for ten successive measurements of a solution containing  $10 \mu mol L^{-1}$  capsaicin with the same electrode and reproducibility was computed for measurements of the same solution carried out with three independent SPCEs. The obtained values are collected in Table 1. A good repeatability (7.9 %RSD) and reproducibility (8.6 %RSD) was achieved, demonstrating the effectivity of the proposed washing step and normalization of the signals, respectively.

**Table 1**

Analytical performance for the cyclic voltammetric determination of capsaicin using a commercial SPCE, a modified rGO-SPCE and commercial SPCNTE, SPCNFE and SPGHE. All measurements were performed in 0.01 mol L<sup>-1</sup> HCl with 0.1 mol L<sup>-1</sup> KCl and after 5 min accumulation at open circuit potential. Currents were normalized in order to compensate for electrode variability within the same commercial batch. Standard deviations are denoted in parenthesis.

	SPCE	rGO-SPCE	SPCNTE	SPCNFE	SPGHE
<b>1st interval</b>					
Sensitivity (μmol <sup>-1</sup> L)	0.055 (0.002)	0.095 (0.001)	0.061 (0.001)	0.066 (0.001)	0.056 (0.001)
R <sup>2</sup>	0.967	0.999	0.982	0.991	0.996
Linear range (μmol L <sup>-1</sup> ) <sup>a</sup>	4.7 – 25.0	1.1 – 25.0	3.2 – 25.0	2.1 – 25.0	1.3 – 25.0
LOD (μmol L <sup>-1</sup> )	1.4	0.3	1.0	0.6	0.4
<b>2nd interval</b>					
Sensitivity (μmol <sup>-1</sup> L)	0.026 (0.001)	0.0210 (0.0005)	0.025 (0.001)	0.024 (0.002)	0.0241 (0.0004)
R <sup>2</sup>	0.979	0.998	0.0967	0.924	0.995
Linear range (μmol L <sup>-1</sup> )	25.0 – 150.3	25.0 – 150.3	25.0 – 150.3	25.0 – 75.1	25.0 – 150.3
Repeatability (%RSD, n = 10)	7.9	4.1	8.3	8.2	6.1
Reproducibility (%RSD, n = 3)	8.6	5.4	8.4	10.0	8.7

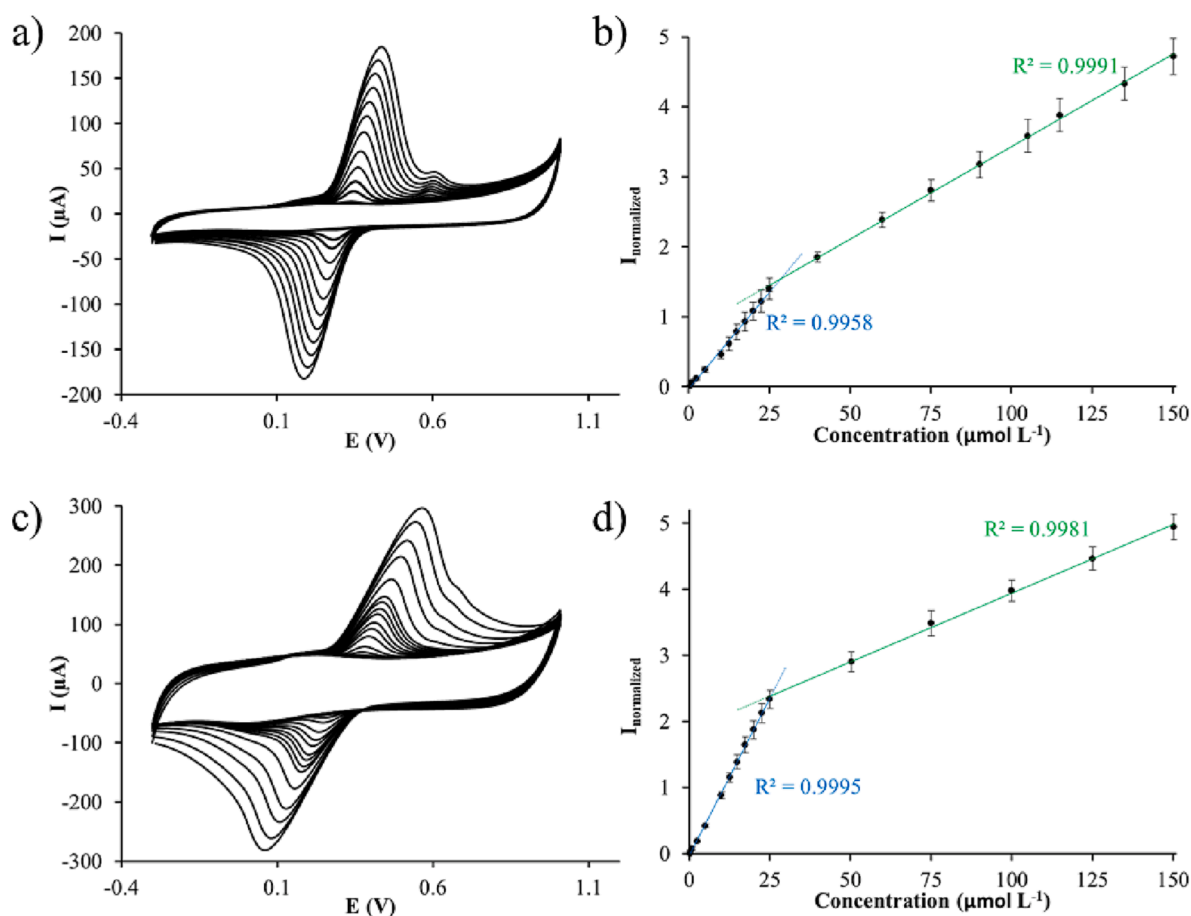
%RSD: relative standard deviation.

<sup>a</sup>The lowest limit of the first linear range was considered as the LOQ.

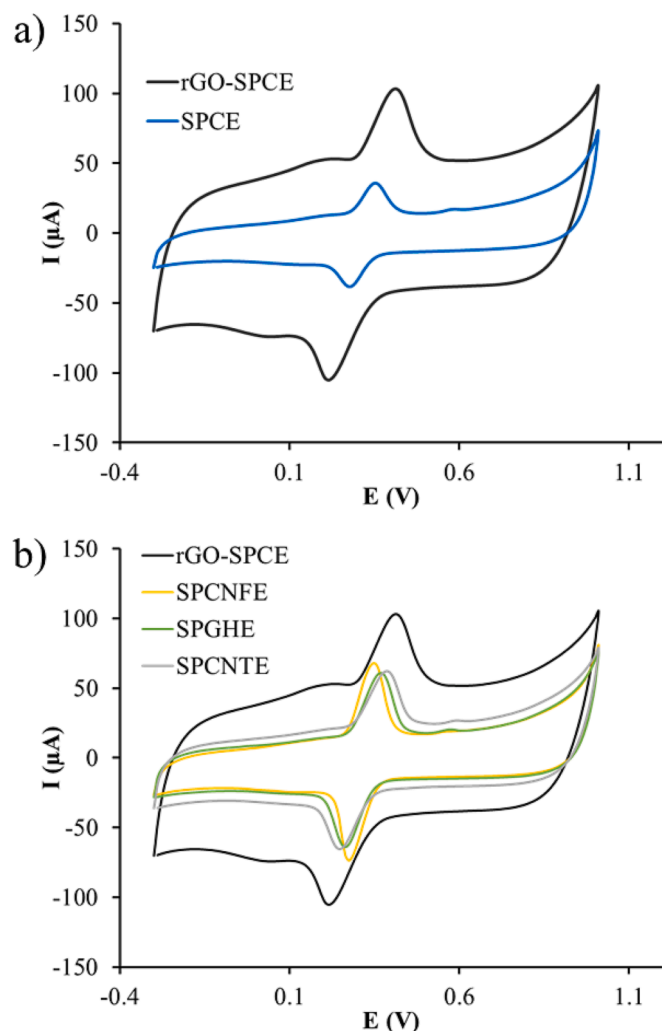
### 3.2. Electrochemical and analytical characterization of rGO-SPCE

The modification of carbon electrodes with rGO has proven to be beneficial in methods where the underlying electrochemical mechanism is adsorption based because it provides an increased surface area with a higher proportion of adsorption sites [22] as well as an enhanced conductivity. In this context, SPCEs were modified with rGO and its benefits for the voltammetric determination of capsaicin were evaluated. The modification was based on the drop-casting of graphene oxide followed

by electrochemical reduction, which was confirmed by a diminishing reduction peak at ca. -0.4 V (Fig. S1). Using the modified rGO-SPCE and applying the experimental conditions optimized for the unmodified SPCE to the determination of 15 μmol L<sup>-1</sup> capsaicin, the obtained voltammetric signal showed a similar shape as that observed for the unmodified SPCE but with a higher predominance of peak I vs peak III. This is related to a higher adsorption due to its larger surface area which, coupled to an enhanced conductivity provided by rGO, is also translated in a higher oxidation current, with an intensity for peak I 2-folds higher



**Fig. 3.** Voltammetric signals (a,c) and corresponding calibration plots (b,d) for increasing concentrations of capsaicin in the range 0.1 – 150 μmol L<sup>-1</sup> recorded with a bare SPCE (a,b) and a modified rGO-SPCE (c,d). CV measurements were performed in 0.01 mol L<sup>-1</sup> HCl with 0.1 mol L<sup>-1</sup> KCl, with a scan rate of 100 mV s<sup>-1</sup> and an accumulation time of 5 min. For error bars n = 3.



**Fig. 4.** Cyclic voltammograms of a solution containing  $15 \mu\text{mol L}^{-1}$  capsaicin in  $0.01 \text{ mol L}^{-1}$  HCl with  $0.1 \text{ mol L}^{-1}$  KCl using (a) SPCE and rGO-SPCE, (b) rGO-SPCE and commercially available SPEs based on carbon nanoallotropes. All measurements were carried out with 5 min of accumulation and at  $100 \text{ mV s}^{-1}$ .

as compared to the unmodified SPCE (Fig. 4a). The increase in capacitive current observed for rGO-SPCE has previously been reported for electrodes modified by drop-casting with graphene [26]. The increase in the voltammetric signal of capsaicin also translated in higher sensitivity for both linear ranges, and a lower LOD (Table 1). It is worth noting that no change was observed in the linearity and saturation of the electrodes, which yields the developed modified electrode suitable both for the determination of low levels of capsaicin detected, for example, in rapeseed or blending waste oils, and for the determination of higher levels of capsaicin in pepper and pepper-based food products without the need of multiple dilution steps. Furthermore, excellent repeatability and reproducibility were also achieved with rGO-SPCE (4.1 %RSD and 5.4 %RSD, respectively), demonstrating, on the one hand, the usefulness of the proposed washing protocol in eliminating capsaicin in electrodes with higher surface area and, on the other hand, the reproducibility of the modification process.

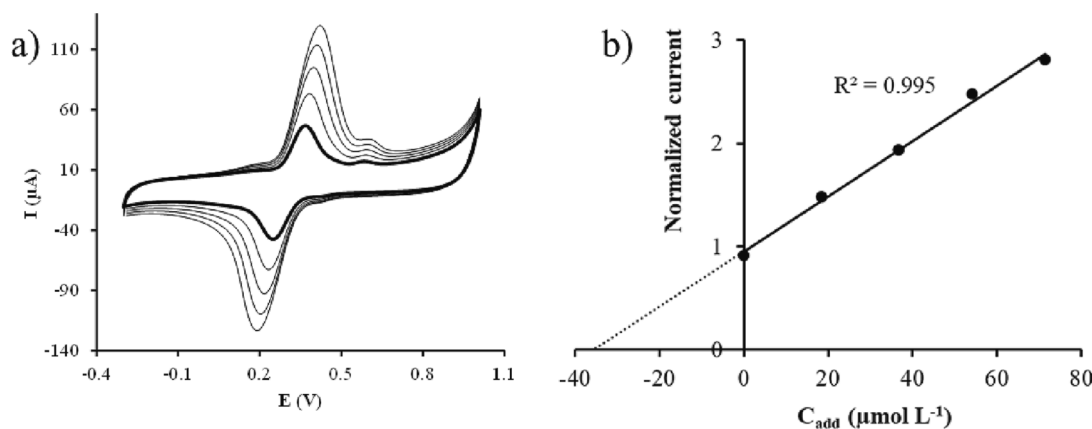
The voltammetric and analytical performance of rGO-SPCE was also compared to commercial SPEs based on carbon nanoallotropes, namely carbon nanotubes, graphene and carbon nanofibers. These electrodes are denoted as SPCNTE, SPGPHE and SPCNFE, respectively, and also provide an increase in surface area, which will favour the voltammetric determination of capsaicin as compared to the simpler SPCE. As it can be observed in Fig. 4b, both SPCNTE and SPGPHE provide smaller

oxidation and reduction peaks for capsaicin whereas SPCNFE displays narrower peaks with a slightly lower intensity. In terms of analytical performance (Table 1), the three commercially available modified electrodes presented slightly higher sensitivities for the first linear range as compared to the unmodified SPCE, but lower than that provided by rGO-SPCE. This highlights the better suitability of rGO for the determination of low levels of capsaicin, most probably related to a larger active area achieved by the drop-casting modification approach. Regarding the second linear range, similar sensitivities were obtained for all electrodes, although in the case of SPCNFE the electrode surface was saturated at lower concentration levels, which resulted in a narrower linear range. It should be noted that all modified electrodes presented slightly lower LODs as compared to the bare SPCE, but no significant change was observed among them. This fact has also been observed in the comparison of several types of carbon electrodes [27]. Furthermore, good repeatability values were obtained for all modified electrodes, which demonstrates the usefulness of the proposed washing protocol regardless of the type of carbon electrode considered. As compared to other voltammetric electrodes reported in the literature for capsaicin (Table S1), the developed rGO-SPCE is a simple and economic alternative that presents one of the wider linear ranges with a fast accumulation time and comparable LOD.

### 3.3. Sample analysis

The applicability of rGO-SPCE sensor for the voltammetric determination of capsaicin was assessed in food samples commercialised in local supermarkets. Specifically, two different branches of paprika (La Vera and a supermarket white-label brand), one red chilli pepper and one Tabasco sauce were analysed. The standard addition method was applied for the voltammetric determination of capsaicin. Then, CV measurements following the above stated conditions were performed including additions of the considered analyte. A whole replicate was measured using the same rGO-SPCE unit.

Representative cyclic voltammograms achieved in the analysis of chilli pepper sample using rGO-SPCE are displayed in Fig. 5a. Well-defined oxidation and reduction peaks were obtained for capsaicin. The standard addition plot for capsaicin (Fig. 5b) illustrates the good correlation of the representative CV measurements done on rGO-SPCE. Similar cyclic voltammograms and calibration plots with good correlation were also obtained by the rest of the studied samples. Table 2 summarizes the total concentrations of capsaicin attained from the CV determination of three replicates of the four considered food samples carried out using rGO-SPCE. As it can be observed, the concordance between capsaicin concentrations from the three replicates was quite good for all studied food samples. In order to evaluate the trueness of the developed CV method, the results obtained by CV were compared with those achieved by HPLC-UV as one of the most widely employed analytical techniques for the determination of capsaicin in food samples (Table 2). In all samples, the concentrations of capsaicin determined by CV were similar to those achieved by HPLC-UV. In order to verify whether the differences in results regarding capsaicin concentration are statistically significant, or not, a statistical analysis with the Excel ANOVA tool was carried out. In the analysis by two-way ANOVA, both the analytical techniques (HPLC-UV and CV) and the studied samples (chilli pepper, paprika La Vera, paprika white-label brand and Tabasco sauce) were compared based on the capsaicin concentration values obtained by each of the three replicates of each sample. Focusing on the comparison of methods, with a 95% confidence level, it can be concluded that there are not significant differences between methods ( $F = 0.028$ ,  $F_{\text{crit}} = 4.49$ , degrees of freedom = 1 and  $\alpha = 0.05$ ). On the other hand, in the comparison between samples, with a 95% confidence level, it can be confirmed that there are indeed significant differences between samples ( $F = 1017$ ,  $F_{\text{crit}} = 3.24$ , degrees of freedom = 3 and  $\alpha = 0.05$ ). This fact corresponds perfectly with what could already be deduced from the analysis of the capsaicin concentration values of each



**Fig. 5.** (a) Representative cyclic voltammograms in chilli pepper sample using a rGO-SPCE with 0.01 M HCl and 100 mM KCl as buffer solution and applying a scan rate of  $100 \text{ mV s}^{-1}$  with a 5 min accumulation time; (b) Capsaicin standard addition plot.

**Table 2**

Total concentrations of capsaicin determined in food samples by cyclic voltammetry (CV) on rGO-SPCE and high-performance liquid chromatography (HPLC). Standard deviations are denoted in parenthesis.

Samples	CV		HPLC	
	c ( $\mu\text{g g}^{-1}$ )	RSD (%)	c ( $\mu\text{g g}^{-1}$ )	RSD (%)
Chilli pepper	2500 (200)	7.3	2500 (200)	7.5
Paprika (La Vera)	89 (6)	6.4	85 (2)	2.6
Paprika (white-label brand)	100 (7)	7.4	86 (4)	4.3
Tabasco™ sauce	119 (4)	3.8	111 (3)	2.7

RSD: relative standard deviation.

n = 3 for RSD (%).

of the studied samples and, from the Scoville test which attributes different SHU values to them. Finally, analyzing the possible interactions between analytical techniques and samples, it can be stated that, with a 95% probability, there is no interaction between the analytical techniques and the samples ( $F = 0.00569$ ,  $F \text{ crit} = 3.24$ , degrees of freedom = 3 and  $\alpha = 0.05$ ).

The good results obtained demonstrate that rGO-SPCE can be successfully used for the CV determination of capsaicin in food samples at different concentration levels and with different matrix complexity resulting, therefore, in an excellent (and more economical) alternative to most conventional analytical techniques such as HPLC.

#### 4. Conclusions

In this work, the voltammetric determination of capsaicin was first studied using an unmodified SPCE, assessing the oxidation–reduction mechanism and concluding that adsorption of capsaicin on the carbon surface plays an essential role. The adsorption of capsaicin highly affects the repeatability and reusability of carbon electrodes, which can be counteracted by the introduction of a simple and effective washing step. Nevertheless, the adsorption of capsaicin also opens the possibility to significantly improve the sensitivity of the method, both by the application of an accumulation time prior to the voltammetric measurement and the use of electrodes with a higher surface area. In this sense, rGO-SPCE was proposed as a better alternative that not only increased the sensitivity as compared to the unmodified SPCE but also as compared to commercially available electrodes based on carbon nanoallotropes. Furthermore, the applicability of rGO-SPCE in real samples was demonstrated through the determination of capsaicin in chilli pepper, red pepper spices and Tabasco sauce, obtaining comparable values to HPLC with UV–vis detection, which was considered as the reference analytical technique.

#### CRediT authorship contribution statement

**Ivet Jiménez:** Methodology, Formal analysis, Investigation. **Clara Pérez-Ràfols:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Visualization, Supervision. **Núria Serrano:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Visualization, Supervision. **Manel del Valle:** Methodology, Validation, Visualization, Writing – review & editing, Resources. **José Manuel Díaz-Cruz:** Methodology, Validation, Visualization, Writing – review & editing, Resources.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.microc.2023.108757>.

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