Guaicolic spices curcumin and capsaicin electrochemical oxidation behaviour at a glassy carbon electrode

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The electrochemical behaviour of the spice biomarkers, curcumin and capsaicin, two polyphenolic compounds with a large spectrum of medical application, has been studied by cyclic, differential pulse and square wave voltammetry at a glassy carbon electrode. The oxidation of curcumin is an irreversible process that in acidic and mild alkaline supporting electrolytes proceeds in two steps. The first irreversible oxidation step leads to the formation of a catechol moiety, and the second reversible step occurs for a higher potential. The oxidation mechanism of ferulic acid, capsaicin and dihydrocapsaicin, curcumin chemical analogues was also investigated. The oxidation of ferulic acid is similar to curcumin whereas the oxidation of capsaicin and dihydrocapsaicin led to the formation of only one oxidation product. A redox mechanism for curcumin oxidation has been proposed.

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1. Introduction

Curcumin, 1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadie-no-3,5-dione, Scheme 1A, and capsaicin, 8-methyl-N-vanillyl-6-nonenamide, Scheme 1C, are the major phytochemicals found in some of the most consumed dietary spice ingredients turmeric (saffron) and chili peppers. Besides the flavour and taste that makes these spices very popular especially in Indian and Mexican cuisine, the curcuminooids from saffron and curry are also popular as colouring agents and food dyes, whereas capsaicinoids from chili peppers are particularly appreciated for the pungent action [1–3].

Due to high medicinal potential with virtually no side effects, these compounds have attracted considerable interest in recent years [1–4]. Their therapeutic potential is being explored in inflammatory [5,6], cardiovascular [7,8], neurodegenerative [9,10], neoplastic diseases [11,12], as well as other disorders [13,14]. The presence of the 4-hydroxy-3-methoxy phenyl residue allows them to specifically interact with sensory neurons, and from this point of view, capsaicin has been used to treat peripheral painful conditions and neuropathies [10,15,16]. It was shown that curcumin regulates the classical and the alternative pathway of nervous system, being used in the treatment of Alzheimer, multiple sclerosis and dementia [9,10,17].

In fact, capsaicin and especially curcumin have received considerable attention owing to their antitumor properties, exerting their effects either at the stage of tumorigenesis or in selectively inducing apoptosis in tumor cells [12,18–20].

The complex mechanism of action of curcumin involves various biological targets such as signal transducers and activators, DNA and several kinase enzymes [18–20]. Also, modulation of intracellular redox state is an indirect mechanism since several critical transcription factors control cell cycle, differentiation, stress response and other physiological processes [19–23]. Curcumin has also been shown to potentiate the effect of chemotherapeutic agents [21] and of γ-radiation [22] in cell culture, and capsaicin is able to trigger apoptosis in human cancer cells, and a direct inhibitory effect of tumour growth has been observed [19,23].

The presence of 4-hydroxy-3-methoxy phenyl residue also confers to curcumin and capsaicin, strong antioxidant activity which leads to radical scavenging ability, also useful to prevent cancer and other mentioned diseases [1–6,24,25]. However, as redox agent, they can also act as pro-oxidants thus exhibiting dual effects on carcinogenic and mutagenic processes [25,26]. Such behaviour is determined by the same structural patterns and perhaps is the explanation for all the controversy surrounding epidemiologic studies about the therapeutic use of phytoantioxidants.

The electrochemical characterisation under different conditions is a promising tool to understand the redox behaviour of polyphenolic compounds in physiological medium and several studies are reported on the electrochemical properties of curcumin [27,28 and...
The aim of the present study is to investigate and propose a mechanism for the oxidative behaviour of the curcumin and capsaicin at glassy carbon electrode, using cyclic, differential pulse and square wave voltammetry, in different pH conditions.

The electrochemical behaviour of the chemical analogues ferulic acid and dihydrocapsaicin was also carried out to clarify and support the proposed mechanism for the oxidative behaviour of the curcumin and capsaicin.

2. Experimental

2.1. Materials and reagents

Curcumin, capsaicin and dihydrocapsaicin from Extrasynthèse (Genay, France) and ferulic acid from Sigma–Aldrich (Spain), were used without further purification. Stock solutions were prepared in ethanol and stored at 4 °C. Solutions of different concentrations of all compounds were prepared by dilution of the appropriate quantity in supporting electrolyte.

All supporting electrolyte solutions [30] were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity < 0.1 μS cm⁻¹), Table 1.

2.2. Apparatus

Voltammetric experiments were carried out using an Autolab PGstat 10 running with GPES 4.9 software, Eco-Chemie, Utrecht, The Netherlands. Measurements were carried out using a three-electrode system in a 0.5 mL one-compartment electrochemical cell (Cypress System Inc., USA). Glassy carbon electrode (GCE, d = 1.5 mm) was the working electrode, Pt wire the counter electrode and the Ag/AgCl (3 M KCl) reference electrode.

The experimental conditions for differential pulse (DP) voltammetry were: pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s⁻¹. For square wave (SW) voltammetry were: pulse of 50 mV, frequency of 10 Hz and a potential increment of 2 mV, corresponding to an effective scan rate of 20 mV s⁻¹, were used.

The GCE was polished using diamond particles of 3 μm (Kemet, UK) before each electrochemical experiment. After polishing, it was rinsed thoroughly with Milli-Q water. Following this mechanical treatment, the GCE was placed in buffer supporting electrolyte and voltammograms were recorded until a steady state baseline voltammograms were obtained. This procedure ensured very reproducible experimental results.

The pH measurements were carried out with a Crison micropH 2001 pH-metre with an Ingold combined glass electrode. All experiments were done at room temperature (25 ± 1 °C) and microliters were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Rainin Instrument Co. Inc., Woburn, USA).

2.3. Acquisition and presentation of voltammetric data

All the voltammograms presented were background-subtracted and baseline-corrected using the moving average application with a step window of 2 mV included in GPES version 4.9 software. This mathematical treatment improves the visualisation and identification of peaks over the baseline without introducing any artefact, although the peak intensity is, in some cases, reduced (<10%) relative to that of the untreated curve. Nevertheless, this mathematical treatment of the original voltammograms was used in the presentation of all experimental voltammograms for a better and clearer identification of the peaks. The values for peak current presented in all plots were determined from the original untreated voltammograms after subtraction of the baseline.

3. Results

The anodic oxidation behaviour of curcumin and capsaicin was investigated at a GCE in different experimental conditions using CV, DP and SW voltammetry, over a wide pH range between 1.0 and 12.0. The electrochemical study of ferulic acid and dihydrocapsaicin was also carried out in order to identify the redox active centres of curcumin and capsaicin.

3.1. Cyclic voltammetry

3.1.1. Curcumin and ferulic acid

On the first CV in 500 μM curcumin in pH = 4.3 0.1 M acetate buffer showed the occurrence of two consecutive anodic processes, peak 1a at $E_{pa1} = +0.57$ V and peak 2a, at $E_{pa2} = +0.67$ V, Fig. 1A.
Changing the scan direction two cathodic processes, peaks 2c,a at \( E_{p2c} = +0.62 \text{ V} \), and peak 3c,a at \( E_{p3c} = +0.34 \text{ V} \), appeared. These two peaks corresponded to the reduction of the curcumin oxidation products formed at the electrode surface during the first voltammetric scan. On the second CV recorded in the same conditions without cleaning the GCE surface, Fig. 1A, a new anodic peak 3a,a at \( E_{p3a} = +0.38 \text{ V} \), occurred. The peaks 3a–3c correspond to a reversible redox reaction and the difference between the anodic and the cathodic potentials \( |E_{p3a} - E_{p3c}| \approx 30 \text{ mV} \) and the current ratio \( I_{p3a}/I_{p3c} \approx 1 \).

At the same time, peak 1a current decreased due to the adsorption of curcumin and/or its oxidation products at the GCE surface.

A new CV experiment was carried out but the scan direction was reversed at +0.62 V, after the occurrence of peak 1a but before peak 2c, Fig. 1A. In this conditions peaks 3a–3c, occurred showing that they are related to the product formed at peak 1a. Recording successive scans in these conditions the current of peaks 3a–3c increased with the number of scans.

The effect of scan rate on the anodic peaks of curcumin was also evaluated. Increasing the scan rate the potential of all peaks were shifted to more positive values. Peak currents were directly proportional to scan rate (v) indicating that the curcumin oxidation was an adsorption controlled process. Nevertheless, a better visualisation of peaks 1a and 2a occurred for v > 100 mV s\(^{-1}\).

Experiments were carried out in supporting electrolytes with different pH values, Table 1, a similar behaviour was observed and peaks 1a and 2a potential were pH-dependent.

CVs at different scan rates in 100 \( \mu \text{M} \) ferulic acid in solutions with 1.3 < pH < 12.0, were performed. Ferulic acid showed a similar behaviour to curcumin and in acetate buffer pH = 4.3 \( 0.1 \text{ M} \), Fig. 1B, on the first anodic scan two consecutive charge transfer reactions, peak 1a, at \( E_{p1a} = +0.51 \text{ V} \), and peak 2a, at \( E_{p2a} = +0.65 \text{ V} \), were observed. Reversing the scan in the negative direction, two reduction peaks 2c,a at \( E_{p2c} = +0.54 \text{ V} \) and peak 3c,a at \( E_{p3c} = +0.31 \text{ V} \), appeared. On the second scan recorded in the same conditions without cleaning the electrode surface, the anodic peak 3a,a at \( E_{p3a} = +0.38 \text{ V} \), occurred. The peaks 3a–3c are due to the reversible redox reaction of ferulic acid oxidation product. At the same time the decrease of peaks 1a and 2a, due to the adsorption of ferulic acid and/or its oxidation product on the GCE surface, was observed.

3.1.2. Capsaicin and dihydrocapsaicin

Capsaicin and dihydrocapsaicin are compounds that mimic the curcumin structure but both have only one ring. The difference between capsaicin and dihydrocapsaicin is the presence of a double bond in the hydrocarbon chain of capsaicin. Nevertheless, this double bond did not interfere in the redox mechanism of either compound and the electrochemical behaviour was very similar, and only the electrochemical study of capsaicin is presented.

CVs in 10 \( \mu \text{M} \) capsaicin in pH = 6.9 \( 0.1 \text{ M} \) phosphate buffer, showed on the first anodic scan peak 1a, at \( E_{p1a} = +0.45 \text{ V} \), and in the reverse scan peak 3a, at \( E_{p3a} = +0.19 \text{ V} \), occurred. Peaks 3a–3c corresponded to the reversible redox processes of capsaicin oxidation product formed on GCE surface, the currents ratio \( I_{p3a}/I_{p3c} \approx 1 \), and the difference between the anodic and the cathodic peaks is \( |E_{p3a} - E_{p3c}| \approx 30 \text{ mV} \). The capsaicin oxidation peak 1a current decreased in successive voltammograms, due to the adsorption of capsaicin oxidation product on electrode surface. CVs were performed in 10 \( \mu \text{M} \) capsaicin in all buffer electrolytes at different scan rates and a similar behaviour was observed.

3.2. Differential pulse voltammetry

The electrochemical behaviour of curcumin, ferulic acid, capsaicin and dihydrocapsaicin, using DP voltammetry, in buffer
supporting electrolytes $1.3 < \text{pH} < 12.0$, was also investigated, Figs. 3 and 4.

On the first DP voltammograms recorded in all electrolytes curcumin and ferulic acid oxidation peaks 1a and 2a, occurred, and the peak currents were higher in acidic media, Figs. 3A and 4A.

For $1.3 < \text{pH} < 9.0$ both peak potentials were pH-dependent and following a linear relationship, Fig. 3B. The slope of the lines, 60 mV per pH unit, indicates that the same number of proton and electron are involved in each redox reactions. The width at half-height of peak 1a, $W_{1/2}$, was close to the theoretical value for the transfer of one electron thus the oxidation of curcumin and ferulic acid involved the transfer of one electron and one proton. For peak 2a, $W_{1/2} \approx 60 \text{ mV}$, the reaction involved the transfer of two electrons and two protons.

For pH $> 9.0$, the oxidation processes was pH-independent, Fig. 3B. The slope of the lines, 60 mV per pH unit indicates that the same number of proton and electron are involved in each redox reactions. The width at half-height of peak 1a, $W_{1/2} \approx 80 \text{ mV}$, was close to the theoretical value for the transfer of one electron thus the oxidation of curcumin and ferulic acid involved the transfer of one electron and one proton. For peak 2a, $W_{1/2} \approx 60 \text{ mV}$, the reaction involved the transfer of two electrons and two protons.

The oxidation of curcumin and dihydrocapsaicin was also pH-dependent, Fig. 4B, on the whole pH interval, and the peak 1a potential variation linear. The slope of the line, 60 mV per pH unit, and the width at half-height of the peak 1a, $W_{1/2} \approx 80 \text{ mV}$, indicated the transfer of one electron and one proton.

DP voltammograms were successively recorded in all electrolytes, Fig. 5. The first DP voltammogram recorded in a solution of 10 \text{\mu M} \text{curcumin}$ showed two consecutive charge transfer reactions, peaks 1a and 2a, Fig. 5A. In subsequent DP voltammograms a new anodic peak 3a appeared at a lower potential and its current increased with the number of scans. This peak is due to the formation of electroactive curcumin oxidation product at the GCE surface. At the same time, the peaks 1a current disappeared in successive DP voltammograms, due to the adsorption of curcumin oxidation products, on the GCE surface, reducing the available electrode surface area. Also, the peak 2a current increased slightly in consecutive DP voltammograms.

The adsorption of the curcumin oxidation products at the GCE surface was confirmed when, at the end of several DP voltammograms in the solution, the electrode was washed with a jet of deionised water and transferred to the supporting electrolyte. The DP voltammogram obtained in these conditions, Fig. 5A, showed peaks 2a and 3a corresponding to the oxidation of the curcumin oxidation products.

Also, the first DP voltammogram recorded in ferulic acid showed both oxidation peaks 1a and 2a, Fig. 5B, and after successive DP voltammograms in the same solution, the oxidation product peak 3a occurred.

DP voltammograms in solutions of capsaicin or dihydrocapsaicin showed only one charge transfer reaction correspondent to peak 1a, after successive DP voltammograms in the same solution, without cleaning the electrode surface, capsaicin peak 3a, Fig. 5C, appeared at a lower potential and the peak current increased with the number of scans.

The pH study of peak 3a was also carried out on the second DP voltammograms in solutions of curcumin, Fig. 6. In each supporting

![Fig. 3](image-url). (A) 3D plots of DP voltammograms base-line corrected in 10 \text{\mu M} \text{curcumin}; (B) plot of ($\bullet$) $E_{\text{pa}1}$ and ($\bullet$) $E_{\text{pa}2}$ vs. pH. Dotted line corresponds to 60 mV per pH unit.

![Fig. 4](image-url). 3D plots of DP voltammograms base-line corrected in 10 \text{\mu M}: (A) ferulic acid and (B) capsaicin.
electrolyte, two consecutive DP voltammograms were recorded and from the second DP voltammogram the peak 3a potential was plotted vs pH. Fig. 6A. Increasing the pH up to 9.0, peak 3a potential decreased, Fig. 6B. The relationship was linear and the slope of the line, ~60 mV per pH unit, shows that the mechanism of peak 3a oxidation process involves the same number of electrons and protons. Considering that $W_{1/2}$ ~ 60 mV, for peak 3a, it was concluded that the oxidation reaction occurs with the transfer of two electrons and two protons. For pH > 9.0, peak 3a potential is pH-independent and the redox reaction involved only the transfer of electrons.

3.3. Square wave voltammetry

SW voltammograms were obtained in 10 μM curcumin in pH = 4.30.1 M acetate buffer, Fig. 7, oxidation peaks 1a and 2a occurring on the first scan, Fig. 7A, showing similar features to the results using CV and DP voltammetry.

Since in SW voltammetry the current is sampled in both positive and negative-going pulses, peaks corresponding to the oxidation and reduction of the electroactive species at the electrode surface can be obtained in the same experiment. Thus, by plotting the forward and backward components of the total current, the irreversibility of peak 1a was observed since there was no backward peak current corresponding to the forward peak current. The reversible character of peak 2a was observed, since forward and backward components occurred with the same current and at the same potential.

On the second SW voltammogram recorded in the same conditions without cleaning the electrode surface, Fig. 7B, peak 3a occurred, due to curcumin oxidation products formed during the first SW voltammetric scan. The deconvolution of the total current showed the reversible character of this redox reaction.

4. Discussion

The electrochemical behaviour of curcumin, ferulic acid, capsaicin and dihydrocapsaicin was investigated using CV, DP and SW voltammetry. The CV study was very important as it enabled rapid screening of the electron transfer processes showing the formation of electroactive products. SW voltammetry was more sensitive than CV and allowed the clarification of the reversibility of the electron transfer processes. DP voltammetry enabled the study of the coupled electron/proton transfer reactions, the formation of electroactive products, and their pH dependence.
All investigated compounds, curcumin, ferulic acid, capsaicin, and dihydrocapsaicin, have a hydroxyl group in the benzene ring, Scheme 1, which is oxidised at peak 1a, Figs. 1 and 2, involving the formation of a phenoxy radical that undergoes hydrolysis usually at ortho- and para- positions, and the hydroxyl groups are immediately oxidised [31,32].

At curcumin oxidation peak 1a occurs the formation of the phenoxy radical, which undergoes hydrolysis at the ortho-position, and the two hydroxyl groups in the benzene ring of an ortho-quinone moiety electrochemically generated are immediately oxidised giving rise to an oxidised product that is reduced at peak 3c, Fig. 1A, when the scan direction was reversed immediately after peak 1a. The ortho-quinone moiety product of curcumin first oxidation, Scheme 2, is reduced, peak 3c, and reversibly oxidised, peak 3a, Figs. 1, 2 and 7. The reversible redox reactions, peaks 3a–3c, were also always observed for ferulic acid, capsaicin and dihydrocapsaicin.

Curcumin and ferulic acid showed on the first voltammetric scan a second oxidation peak 2a, Figs. 1, 3A, 4A and 5. This redox reaction is absent on the voltammograms in solutions of capsaicin, Figs. 2 and 4B, and dihydrocapsaicin indicating that peak 2a is related with the double bond of the hydrocarbon chain of the curcumin and ferulic acid molecules.

For a higher potential curcumin and ferulic acid oxidation peak 2a is due to an oxidation, after hydroxylation at position 1 and/or 7, involving the formation of a product that undergoes reversible redox reactions, peaks 2a–2c, Scheme 2, as showed by SW voltammetry for curcumin Fig. 7, and by CV for curcumin, Fig. 1A, and ferulic acid, Fig. 1B.

5. Conclusion

The electrochemical behaviour of curcumin, a compound with a large spectrum of potential medical applications was investigated, the oxidation is pH-dependent and occurs in two consecutive oxidation steps. The chemical analogues ferulic acid, capsaicin and dihydrocapsaicin electrochemical behaviour was also investigated. The oxidation of ferulic acid is similar with that of curcumin involving two consecutive oxidation steps, whereas the oxidation of capsaicin and dihydrocapsaicin involve only one oxidation step. An oxidation mechanism for curcumin was proposed.
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