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DETERMINATION OF QUERCETIN AND LUTEOLIN IN PAPRIKA SAMPLES BY VOLTAMMETRY AND PARTIAL LEAST SQUARES CALIBRATION

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Abstract

Quercetin and luteolin are flavonoids with beneficious properties, which are present in paprika. In this work, both have been determined in paprika by using electrochemistry combined with chemometrics. The electrochemical oxidation mechanisms of both analytes have been studied through sampled direct current (DC) voltammetry, differential pulse voltammetry (DPV) and Square Wave Voltammetry (SWV), making use of a glassy carbon electrode. The final technique selected for the quantification was DPV due to its high repeatability with respect SWV. The chemical variables and the instrumental parameters were optimized and the final conditions employed were ethanol: water (20:80), 0.75 mol dm^{-3} of HCl, and a pulse amplitude of 50 mV. Due to the facts that oxidation potential of both analytes were quite similar, their DPV peaks were overlapped, and also because the analytes interaction during the electrochemical process causes a non-additivity of the signals, they could not be quantified separately by direct measurement of peak intensity. For this reason, a chemometric algorithm was applied (partial least squares (PLS) regression in its modality PLS-2). In the case of validation samples, appropriate sets of calibration and validation were built and good results were obtained. This methodology was applied to real paprika samples and the results were similar to those obtained with a HPLC method previously reported.

Keywords: paprika; quercetin; luteolin; differential pulse voltammetry; partial least squares 2 (PLS-2)

1. Introduction

Polyphenols having a benzo-γ-pyrone structure constitute a family of compounds known as flavonoids. Flavonoids are widely distributed among higher plants and foods [1]. Among all of them, paprika is included. It is a spice mostly used in cooking and it presents a high concentration of these compounds, which show antioxidant, anticarcinogenic and antiinflamatory properties [2]. In Spain, this product is obtained mainly in two areas, Murcia and Extremadura. Its use is increasing in the last four years [3]. Some of its components have health benefits for consumers and, for this reason, to know which components are present as well as their respective concentration is important.

Quercetin and luteolin (Figure 1) are the most expressed flavonoids in plants. In vitro studies have shown that quercetin may have positive effects against cancer and neurodegenerative diseases. Regarding to luteolin, it shows important biological activity in plants, which can be exploited in pharmaceutical formulations [4]. Both are mainly present in glycosylated forms. Hydrolysis will remove the glycosylation, thus increasing the yield of quercetin or luteolin aglycone, and simplifying the qualitative and quantitative analysis [5].

Normally, this kind of compounds has been analyzed in paprika or other foods by means of separative techniques combined with different detectors [6]. Separative techniques require more analysis time and volume of solvent than spectroscopic or electrochemical techniques require. Our research group has proposed methods for the determination of quercetin, luteolin and kaempferol in paprika by two different methodologies [7,8]. One of them employing a liquid chromatography method with diode-array and fluorescence detections, and another one using fluorescence and second-order algorithms. Respect to the analysis time, both offered better results than other methods found in the bibliography.

However, taking into account that these compounds present electroactive groups, their analysis by means of electroanalytical techniques has been considered interesting, especially because these

techniques present different benefits, as they are neater, faster, simpler, and the instrumentation is competitively affordable.

In the studies found about the electrochemical behaviour of flavonoid compounds it is shown that quercetin and luteolin present similar oxidation potential when the medium conditions are similar [4,9] and, as a consequence, their voltammetric signals are overlapped. For this reason, it is difficult to quantify them simultaneously without chemometric algorithms.

In the last decades, electrochemical techniques have been combined with chemometric algorithms to quantify binary, ternary or quaternary mixtures of different compounds in different matrices. In a review published the last year, the studies that combined electrochemistry with multi-way calibration are collected [10]. Sometimes, mixtures of compounds can be analyse simultaneously applying first-order algorithms to first-order data. This presents the advantage that the experiments are quicker and the data treatment is easier than when multi-way data and algorithms are used.

Some of the problems addressed in the last decade make use of differential pulse voltammetry (DPV), Ni et al. determined four pesticides in water using classical least squares (CLS), principal component regression (PCR), partial least squares (PLS) and radial basis function-artificial neuronal networks (RBF-ANN) [11], Gui et al. have quantified three 5-nitroimidazoles compounds in foodstuff with PLS, principal component regression (PCR) and radial basis function artificial neural network (RBF-ANN) models [12], Zapata-Urzúa et al. have determined different drugs using partial least-squares (PLS-1) as chemometric algorithm [13] and Gholivand et al. have quantified five opium alkaloids in complex matrices using also PLS [14]. As is observed, PLS is mainly utilized.

Apart of DPV, other electroanalytical techniques have been employed with first-order algorithms. For example, differential pulse adsorptive stripping voltammetry (DPAdSV) has been used to quantify nalidixic acid and its main metabolite [15], metals in bioethanol fuel [16] and antibiotic drugs in food samples [17]. On the other hand, Tonello et al. have carried out the simultaneous

determination of essential oils in honey samples employing square wave voltammetry and PLS-1[18], and Henao-Escobar et al. have quantified biogenic amines using also square wave voltammetry and PLS [19].

With this background, it is demonstrated the extensive use of chemometrics in voltammetric techniques in different fields and matrices.

About the determination of quercetin by electrochemical techniques, studies employing different modalities and electrodes have been found in the last years [20–25]. Also, a method to determine simultaneously quercetin and kaempferol has been developed this year [26]. However, there is no report for simultaneous determination of quercetin and luteolin employing any electrochemical method.

In this study, differential pulse voltammetry and a PLS method are proposed for the resolution of binary mixtures of flavonoid compounds, quercetin and luteolin. The mainly aim of this work is to propose an alternative to the previously developed methods, easy, fast, less expensive and applicable to samples of paprika.

2. Materials and methods

2.1. Chemical reagents and samples

Luteolin standard was purchased from Extrasynthese (Genay Cedex, France). HPLC-grade methanol (MeOH) solvent and quercetin standard were obtained from Sigma (Sigma-Aldrich Química, S.A., Madrid). Ethanol (EtOH) (96%) was supplied by Panreac (Panreac Química, S.A.U., Barcelona). Sodium hydroxide pellets pharma grade and hydrochloric acid, 37%, were obtained from Panreac (Panreac Química, S.A.U., Barcelona). Sep-Pak Plus C18 cartridges of 360 mg were obtained from Waters (Waters Corp., Milford, MA, USA).

Samples of paprika were obtained from local market. They belonged to different origins, to the Spanish Protected Designation of Origin (PDO) "*Pimentón de La Vera*" and other different producers.

Britton-Robinson buffer solutions were prepared containing a mixture of boric acid, orthophosphoric acid and acetic acid 0.04 M and adding different volumes of sodium hydroxide 0.2 M to obtain different pH values.

2.2. Instrumentation and software

An Autolab AUT 12.v PSTAT10 (Ecochemie, The Netherlands) has been used in combination with a Metrohm VA-663 polarographic stand (Herisau, Switzerland). It presents a three-electrode system: glassy carbon electrode (Metrohm 6.1204.040 Ref.) as working electrode, a Ag/AgCl 3M KCl reference electrode and a Pt wire auxiliary electrode. The system is monitored by the General Purpose Electrochemical System version 4.0 (GPES4), software package (Ecochemie, Ultrecht, The Netherlands).

The validation of the proposed analytical method was carried out using an Agilent Model 1100 Liquid Chromatography instrument (Agilent Technologies, Palo Alto, CA, USA), equipped with degasser, quaternary pump, column oven, autosampler Agilent 1260 infinity, UV-visible-diode-array detector (DAD) and fluorescence-detector (FLD). Chemstation software package was used to control the instrument, data acquisition, and data analysis. The analytical column employed was a rapid resolution Zorbax Eclipse XDB-C18 column (4.6 mm x 50 mm x 1.8 µm) (Agilent Technologies).

Calibration curves and analytical figures of merit were obtained by means of the homemade ACOC program [27]. The software package The Unscrambler® v6. 11 (CAMO A/S Olav Tryggvasonsgt, N-7011, Trondheim, Norway) was used for the experimental design and for building PLS models.

2.3. Pre-treatment of the glassy carbon electrode

In order to ensure the repeatability of the glassy carbon electrode surface this was cleaned after each voltammetric experiment rubbing with a cotton soaked in dimethylformamide (DMF) for 2 min, and then with a cotton soaked in water for 1 min. In the case of paprika samples analysis, a

blank solution was registered between different samples to check the effectiveness of clean-up procedure of the electrode surface.

2.4. Electrochemical procedure

The finally selected electrochemical technique was differential pulse voltammetry (DPV). The voltammogram was registered with a scan rate of 10 mV/s and a pulse amplitude of 50 mV, from + 0.45 to + 0.80 V. Baseline correction was performed making use of the moving average option offered in the GPES4 software package.

2.5. Calibration and validation sets for the PLS models

For training the PLS models, a calibration set of sixteen binary mixtures was prepared using a full factorial design with three concentration levels of each analyte in the range 1.0 to 5.5 µg mL^{-1} for quercetin and 0.5 to 5.0 µg mL^{-1} for luteolin. These intervals were selected considering the concentrations expected in the paprika samples.

The standards were prepared in 10.0 mL volumetric flasks. In order to obtain the final conditions selected, the suitable volume of quercetin and luteolin stock solutions, 2.0 mL of EtOH and 2.0 mL of HCl (3.75 mol dm^{-3}) were added.

The validation set was formed by 9 samples, which were prepared with concentrations different from those employed for calibration, but within their corresponding calibration ranges.

2.6. Chromatographic method

The validation of the proposed analytical method was carried out by a HPLC-DAD method previously optimized [7]. The mobile phase consisted in H_3PO_4 (0.03 mol dm^{-3}) in high-purity water (solvent A) and methanol (solvent B), 50:50. The flow rate was set constant at 1.0 mL min^{-1} and the injection volume was 10 µL. The DAD detection was performed at 360 nm for luteolin and at 370 nm for quercetin.

2.7. Paprika sample treatment

The analytes were extracted from precisely weighed aliquots of 0.5 g of paprika sample with 20 mL of methanol for 30 min in an ultrasound bath. The extract solution was centrifuged and the supernatant liquid was evaporated to dryness. The residue was suspended in 50 mL of water and loaded on a C18 cartridge (Solid Phase Extraction), previously conditioned with 8 mL of acetonitrile and 8 mL of water. The cartridge was successively washed with 20 mL of water and 20 mL of 10 % v/v MeOH to eliminate other compounds present in paprika matrix. The analytes were eluted with 2.5 mL of 85 % v/v MeOH. Finally, they were diluted to a final volume of 3.0 mL with 85 % v/v MeOH.

An aliquot of 1.0 mL of the obtained solution was subjected to hydrolysis to obtain flavonoid from their corresponding aglycones. A volume of 2.0 mL of HCl 3.75 mol dm^{-3} was added to obtain a final concentration of 2.5 mol dm^{-3} in the solution for the hydrolysis. This procedure was performed in a water bath at 85 ºC for 45 min. After the hydrolysis step, a volume of 2.0 mL of EtOH was also added and the sample was diluted to the mark with water.

3. Results and discussion

3.1. Electrochemical behaviour

Firstly, cyclic voltammograms were recorded for a standard solution of quercetin and luteolin. Figure 2 shows the cyclic voltammograms in acid medium. It can be observed two well-defined oxidation peaks at + 0.45 V for quercetin and at + 0.6 V in the forward scans. No reduction peak is observed for quercetin in the reverse scan, which indicates the non-reversible character of this electrochemical process. In the case of luteolin, a small reduction peak is observed in the reverse scan, so, the reversibility criteria were applied to study the reversibility of this process as it is indicated in the following sections.

3.1.1. Influence of pH

The study was performed with Britton-Robinson buffer solutions, investigating the influence of pH on the electrochemical behaviour of quercetin and luteolin over the pH range 2 – 8. The voltammograms exhibited a single well-defined sample DC wave in the investigated pH range

(Figure 3). This wave shifted with pH towards less positive potentials indicating the participation of protons in the electrochemical processes. Besides, the intensity of the signal decreases over the pH 5.5 and 6.5 for quercetin and luteolin, respectively, being these pH values near to the first pK values for these compounds found in the literature[4,9]. Over pH 9 -10, the natural oxidation of the analytes is happening as is described in another study employing different techniques [8] and the signals disappear. The results were similar using DPV and SWV. With the aim to secure the highest signal, an acid medium (pH = 2) was selected for the further experiments.

3.1.2. Study of the electrochemical oxidation

In order to show up the electrochemical mechanism of these compounds described in the literature, the number of protons and electrons involved in the oxidation process was calculated.

Firstly, the influence of pH in the $E_{1/2}$ (Fig 1.) was evaluated according to the equation 1 [28], where m and n are the number of protons and electrons interchanged in the oxidation process, respectively, and α is the electronic transfer coefficient.

$$E_{1/2} = C - 0.059 \cdot \left(\frac{m}{n \cdot \alpha}\right) \cdot \mathrm{pH} \qquad (1)$$

In the case of quercetin, from the representation $E_{1/2}$ versus pH, the slope value obtained was 0.0638. Hence, the value $m/n \cdot \alpha$ was 1.08, that is to say, the number of protons are the same that the $n \cdot \alpha$ value.

In order to obtain the value of $n \cdot \alpha$, a similar solution was prepared and its voltammogram was registered in stationary regime (by stirring the solution). A value of $n \cdot \alpha$ of 1.76 was obtained from the logarithmic analysis of this voltammogram. Thus, the value of m is 1.91 and, as consequence, the participation of two protons in the oxidation process is deducted. The same process was followed in the case of luteolin, and the results were the same.

To calculate the number of electrons taking part in the oxidation process, the voltammogram DC of a solution of hydroquinone with same molar concentration of quercetin was registered. The wave of hydroquinone shows limiting current similar to the wave of quercetin. It is known that

hydroquinone interchanges two electrons. Although the diffusion coefficients of quercetin and luteolin probably differ of those of hydroquinone, given these results and taking into account the references found in the literature [4,12], it can be said that quercetin and luteolin interchange two electrons.

3.1.3. Nature of electrochemical processes

To characterize the nature of these oxidative processes, a study about the influence of scan rate over the peak current obtained in linear sweep was conducted. It was found that the plot of the peak current versus square root of scan rate ($v^{1/2}$) is linear over the whole range of the scan rate studied, indicating a typical diffusion-controlled current system. This behaviour is the same for both analytes.

Besides, in order to elucidate the reversible or non-reversible character of these electrochemical processes, a study was made by means of cyclic voltammetry. The cyclic voltammograms of solutions of quercetin (1.65 x 10^{-5} mol dm^{-3}) and luteolin (1.75 x 10^{-5} mol dm^{-3}), prepared with a 20 % of EtOH and at pH = 2, were registered. It is well-known that the requirements of a reversible system must fulfil are $\Delta E = E_{pa} - E_{pc} \approx 0.059/n$ V and $I_{pa}/I_{pc} \approx 1$.

In the case of quercetin, the cathodic wave does not appear, so it claims that the process is non-reversible. However, in the case of luteolin, the cathodic wave appears. In this case, the reversibility criteria were applied and the process was also found to be non- reversible.

3.2. Influence of chemical variables

Due to the convenience of utilizing an ethanolic acid medium to prepare the final sample solutions, the optimization of these variables was performed utilizing the signal obtained by differential pulse voltammetry.

3.2.1. Influence of the ethanolic medium

The volume of EtOH was varied in the range from 5 to 25 % founding that the increase of EtOH caused a decline of the intensity. In the following analysis of these analytes, in the paprika

samples, the possible adsorption of different interferences components from the paprika matrix and the subsequent deterioration of the electrode surface must be taken into consideration. For this reason, with the aim of reducing possible adsorption phenomena of other matrix components, 20 % of EtOH was chosen for the further studies, in spite of the fact that sensitivity is lower.

3.2.2. Influence of HCl concentration

Quantitative determination of individual flavonoid glycosides in plant materials is difficult, due to their large number. Therefore, the glycosides are normally hydrolysed and the resulting aglycones are identified and quantified. Moreover, their oxidation potential are very similar, which can difficult their electroanalytical determination [20].

For this reason, a previous developed method of extraction and hydrolysis was employed [7]. In this method, a strongly acid medium was necessary. For this reason, the influence of HCl concentration was studied. The concentration was assayed between 0.4 and 1.5 mol dm^{-3} and it was observed that the HCl concentration did not affect the analytical signal. Hence, the concentration chosen was 0.75 mol dm^{-3} to prepare the standard solutions which was consistent with the concentration resulting of carrying out the previous hydrolysis procedure of flavonoid glycosides of quercetin and luteolin in the case of the paprika samples.

3.3. Influence of instrumental parameters

In this study, two electrochemical techniques, differential pulse voltammetry and square wave voltammetry (SWV), were taken into account for the quantification of flavonoid compounds because they presented more sensitivity that sampled DC and cyclic voltammetry.

The pulse amplitude, ΔE, employed in DPV, was varied between 10 and 100 mV and both, peak intensity (I_p) and peak width, increased linearly with this variable. A value of 50 mV in the pulse amplitude was selected as optimum value to avoid an excessively width peak that favours the signals' overlapping.

In SWV, the frequency was varied between 20 and 100 Hz. Finally, 80 Hz was selected as optimum to avoid an excessively width peak as in the previous case.

Between both techniques examined, the technique used for analytical purpose was chosen by comparing their repeatability. Table 1 shows the values obtained in each case. It is observed that relative standard deviation (RSD) was better in the case of DPV, which was consequently the selected technique.

3.4. Analytical parameters

The influence of the concentration of these compounds was studied by DPV with standards prepared in duplicate, under the selected optimal conditions, between 0.5 and 20 µg mL^{-1} of quercetin and between 0.25 and 12 µg mL^{-1} of luteolin. The voltammograms were registered between 0.45 and 0.85 V, utilizing a pulse amplitude of 50 mV and a scan rate of 10 mV/s. The results of the linear univariate analysis of peak height are presented in the Table 2 and the corresponding calibration curves are shown in the Figure 4. It can be said that good values of linearity and detection limits [29] were obtained.

Besides, the evaluation of the precision of the method was performed by carrying out the analysis of several standard solutions containing 4.0 µg·mL^{-1} of quercetin and luteolin in the same day (intra-day precision, n = 5), and containing 5.0 µg·mL^{-1} of each one in different days, during 5 days (inter-day precision). The RSD values of the peak intensities were 2.2 and 2.5 % for quercetin and luteolin, respectively, in the same day, and 2.6 and 3.2 % for quercetin and luteolin, respectively, in different days. These results show a good precision of the method.

3.5. Simultaneous determination of quercetin and luteolin

At first, with the aim of analysing the two compounds simultaneously, the behaviour of standard mixtures of quercetin and luteolin was examined. As appreciated in Figure 5, although I_p of quercetin could be measured without interference from luteolin, for the determination of this last compound a system of equations may be necessary. However, an additional problem of non-additivity of signals occurs, and the voltammogram corresponding to the mixture of flavonoids

(Figure 5) is quite different from the one obtained by summing the voltammetric signals of the two single compounds, registered according to the section 2.4.

The non-additivity cannot be explained by chemical interaction between these species in solution that may affect the chemical nature of these compounds, because this mixture has been analyzed by means of other techniques (chromatography and fluorescence) appearing unaltered signals of both analytes. Therefore, the non-additive character of these electrochemical signals must be attributed to another kind of interaction that affect the electrochemical process. Hence, a change in the shape of the waves in the DC voltammogram is observed when they are in presence one of the other, even though these peaks are still distinguishable.

From these results, it was decided to make use of the method Partial Least Squares (PLS). This "full-voltammogram" method assumes a linear relationship between the measured signal (intensity for each value of potential) and the concentrations of all the components in the mixture. However, it has provided good results also with non-linear systems, when secondary chemical reactions occur, or systems in which non-additive signals occur [30].

In this study, the two modalities of PLS (PLS-1 and PLS-2) were assayed and the statistical parameters of both models are compared in the Table 3. With these models, a set of synthetic samples corresponding to different mixtures of quercetin and luteolin standards were predicted. The relative error of predictions (REP) were calculated and they were 36.7 % (PLS-1) and 12.3 % (PLS-2) for quercetin, and 13.4 % (PLS-1) and 12.8 % (PLS-2) for luteolin. It can be said that PLS-1 and PLS-2 offered similar results in the case of luteolin, however, in the case of quercetin, better results were obtained for PLS-2. For this reason, it was decided to use it for the prediction of real paprika samples.

3.6. Paprika samples analysis

The optimized model was used to obtain the concentration of quercetin and luteolin in different paprika samples. For the validation of the proposed analytical method, a HPLC method described in the literature was used [7].

The determination was carried out by extracting the analytes from paprika samples as it is described in the section 2.7. Figure 6 shows a voltammogram of a paprika sample and a standard solution of a mixture of both analytes presenting similar shape. The samples were analyzed in duplicate and the results were compared with those obtained by HPLC. Results are in the Table 4 showing in general good agreement.

These results are reinforced by the elliptical join confidence region (EJCR) test [31], which computes the joint confidence interval for the intercept and the slope of the found versus nominal (DPV-PLS-2 method versus HPLC method in this case) concentration plot, and check if the ideal values of 0 and 1 are within the ellipse (Figure 7). The result of EJCR test offered a good correlation between both methods as it complies the test.

4. Conclusions

In this paper, the electrochemical behaviour of quercetin and luteolin are presented. The interchange of two protons and two electrons in their electrochemical oxidation has been demonstrated.

After observing that both oxidation potentials were similar and the corresponding oxidation peaks were overlapped, it was decided to develop a methodology combining electrochemistry with chemometrics for their joined analysis. In order to obtain the better signal and apply the methodology to real paprika samples, the chemical variables and the instrumental parameters were optimized.

Finally, the electrochemical technique which offered better results was differential pulse voltammetry and the algorithm employed was PLS in the modality PLS-2. The results were good in the case of validation samples for both analytes. For the real samples, the methodology was validated by a HPLC method and the results were also good.

It must be noticed that this method presents the advantage of shorter analysis time than in the case of separative techniques, thanks to the use of chemometrics. Besides, much less solvent is utilized for the analysis, being the new method more respectful to the environmental.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

Figure captions

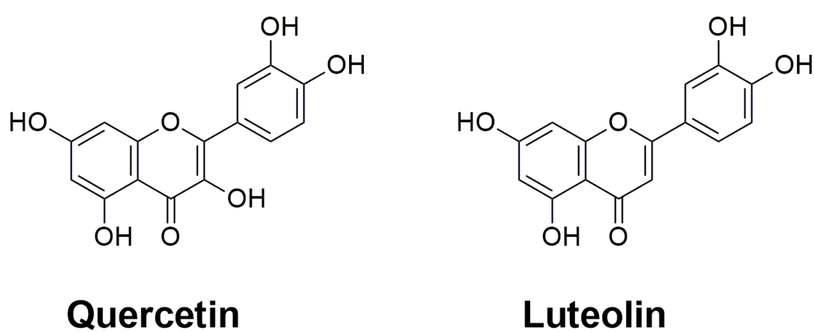

Figure 1. Chemical structures of quercetin and luteolin.

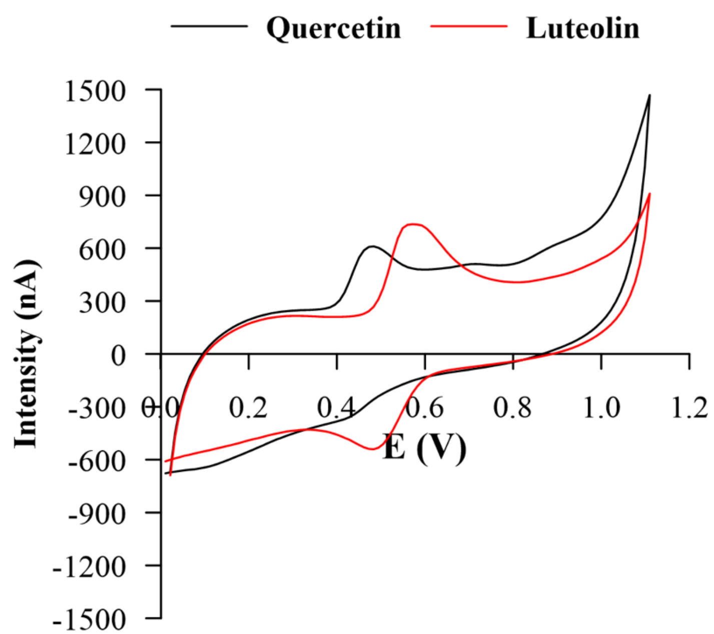

Figure 2. Cyclic voltammograms obtained for a standard solution of 5.0 µg mL^{-1} (1.65×10^{-5} mol dm^{-3}) of quercetin and for a standard solution of 5.0 µg mL^{-1} (1.75×10^{-5} mol dm^{-3}) of luteolin at pH = 2.

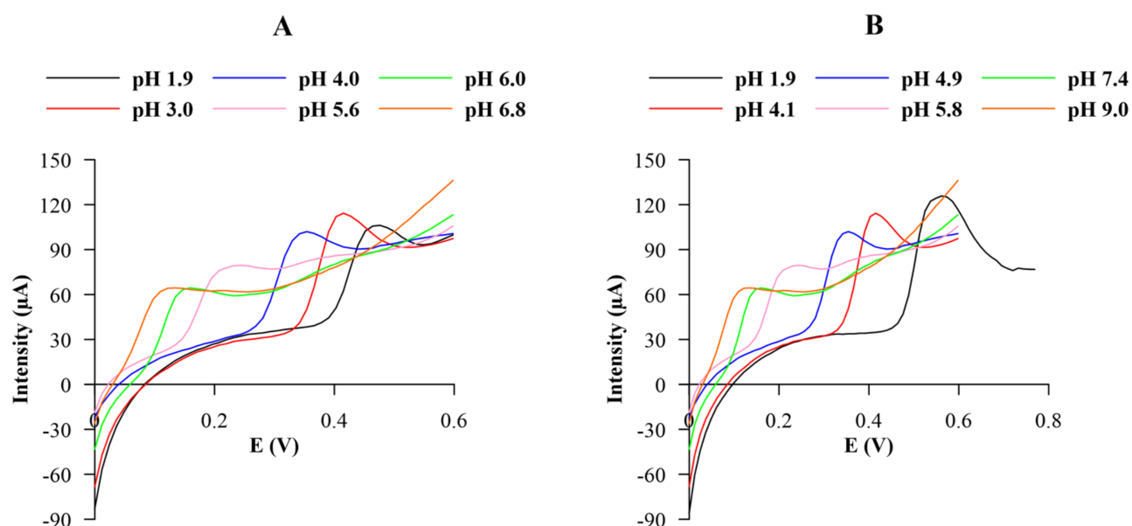

Figure 3. DC voltammograms obtained for a standard solution of 5.0 μg mL^{-1} (1.65 x 10^{-5} mol dm^{-3}) of quercetin (A) and for a standard solution of 5.0 μg mL^{-1} (1.75 x 10^{-5} mol dm^{-3}) of luteolin at different pH values.

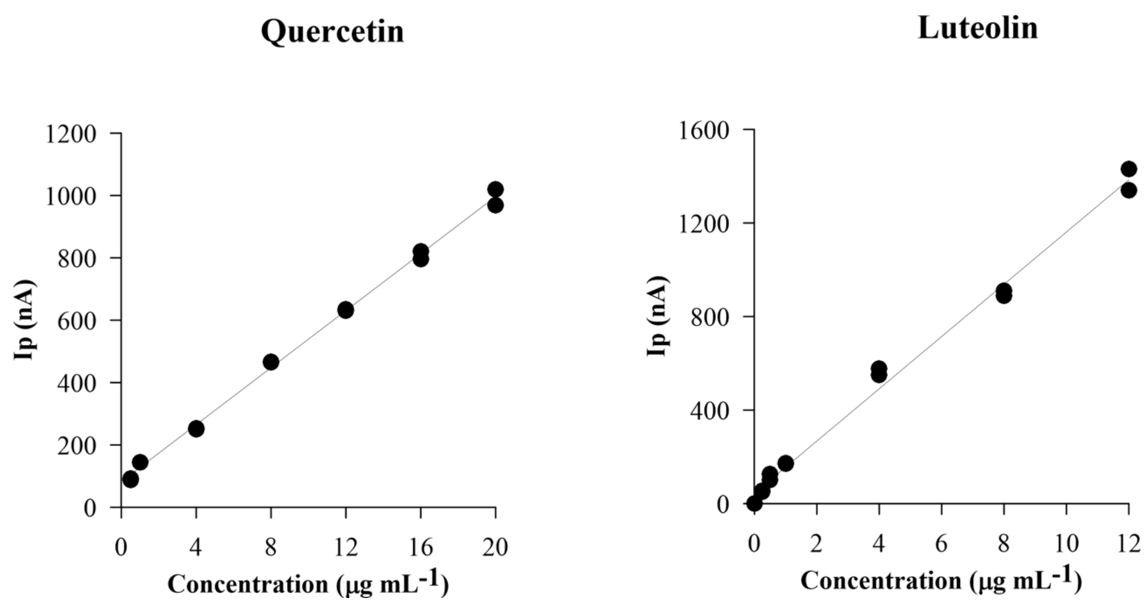

Figure 4. Calibration curves obtained for quercetin (A) and luteolin (B) with the analytical method proposed.

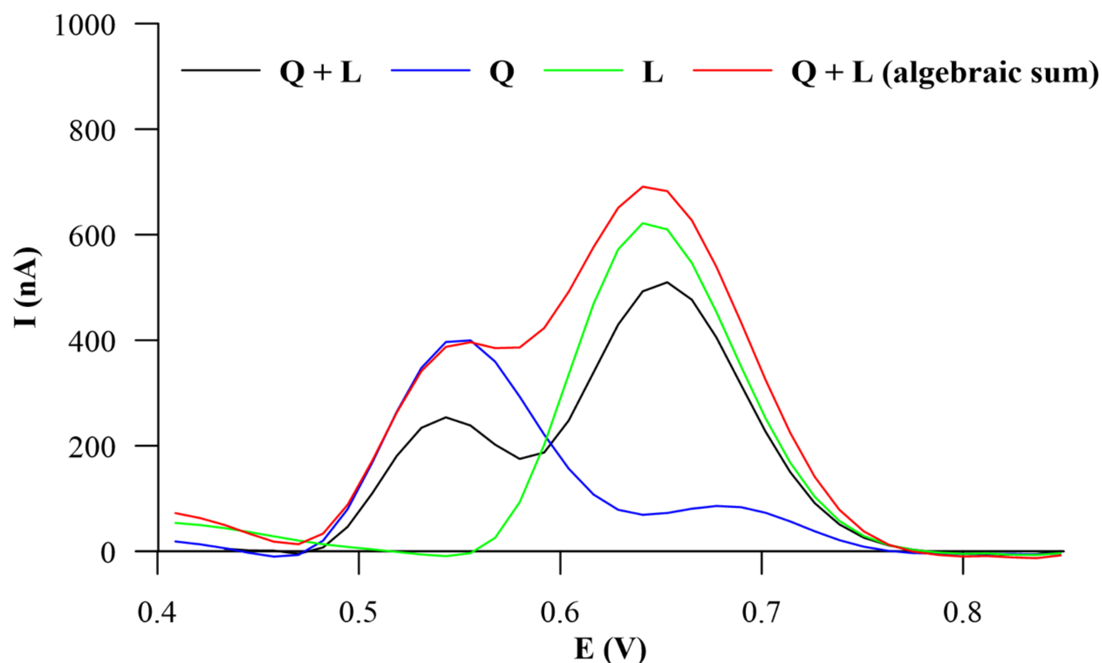

Figure 5. DPV voltammograms of a standard mixture of quercetin and luteolin of 5.0 μg mL^{-1} each one (black line), quercetin standard of 5.0 μg mL^{-1} (1.65 x 10^{-5} mol dm^{-3}) (blue line), luteolin standard of 5.0 μg mL^{-1} (1.75 x 10^{-5} mol dm^{-3}) (green line) and algebraic sum of the signals of the quercetin and luteolin standards (red line).

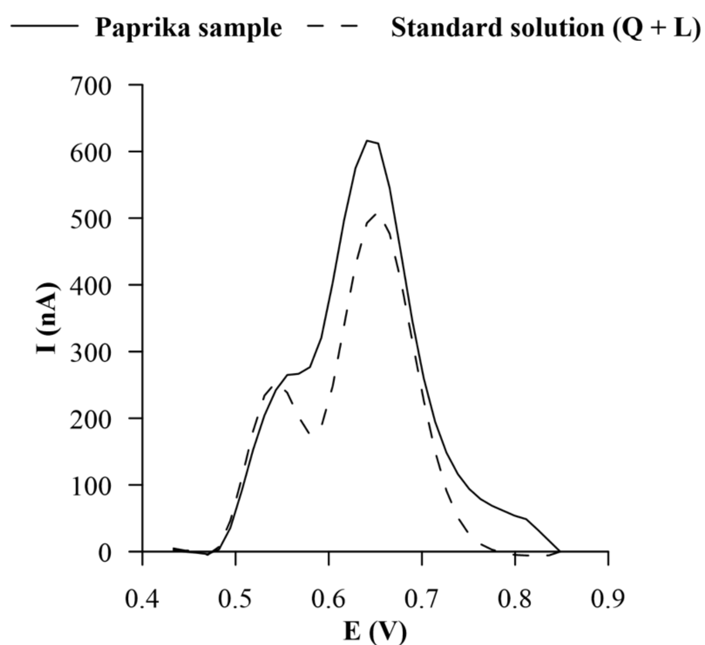

Figure 6. DPV voltammogram obtained for a paprika solution and a standard containing 2.0 μg mL^{-1} of quercetin (0.66 x 10^{-5} mol dm^{-3}) and luteolin (0.70 x 10^{-5} mol dm^{-3}).

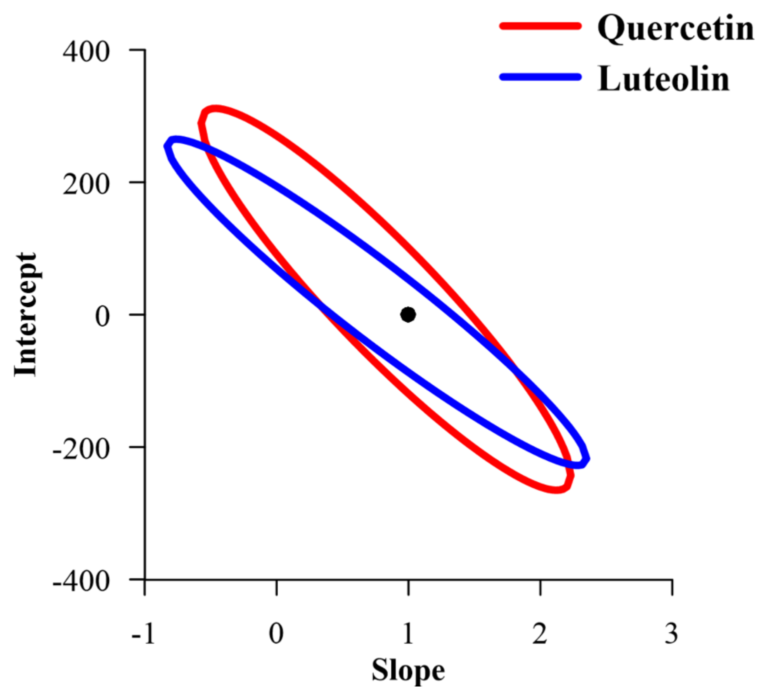

Figure 7. Elliptical join confidence region (EJCR, 95 % confidence level) for the slope and intercept of the regressions of the concentration predicted by PLS and those calculated by HPLC.

Table 1. Relative standard deviations obtained from the Ip measured in square wave and differential pulse voltammograms of the analytes.

	Quercetin		Luteolin	
	SWV (I_p, μA) (1 μg mL^{-1})	**DPV (I_p, μA) (4 μg mL^{-1})**	**SWV (I_p, μA) (1 μg mL^{-1})**	**DPV (I_p, μA) (4 μg mL^{-1})**
	0.568	0.410	1.42	0.644
	0.575	0.393	1.44	0.638
	0.650	0.391	1.15	0.614
	0.862	0.392	1.40	0.612
	0.595	0.403	1.39	0.637
RSD (%)	19	2.2	8.7	2.5

Table 2. Analytical parameters of the proposed DPV method.

Analyte	Lineal range (μg mL^{-1})	Intercept ± SD	Slope ± SD (nA mL μg $^{-1}$)	Correlation coefficient (r)	Linearity (%)	LOD[a] (μg mL^{-1})
Quercetin	0.5 – 20	84.5 ± 9.1	45.5 ± 0.7	0.998	98.6	0.5
Luteolin	0.25 - 12	44.5 ± 15.7	111.7 ± 2.8	0.996	97.6	0.42

SD: Standard Deviation
[a]LOD: Limit of detection according to Long and Winefordner [29]

Table 3. Statistical parameters corresponding to the different models applied

	Quercetin		Luteolin	
	PLS-1	**PLS-2**	**PLS-1**	**PLS-2**
N	2	2	2	2
RMSEC	0.6690	0.5882	0.5245	0.6722
R^2	0.965	0.973	0.973	0.965

N: principal components determined by the model
RMSEC: Root-mean-square error of calibration
R^2: determination coefficient

Table 4. Results obtained for real paprika samples

Sample	Quercetin (mg kg^{-1} wet paprika)* ± SD		Luteolin (mg kg^{-1} wet paprika)* ± SD	
	DPV – PLS-2	**HPLC**	**DPV – PLS-2**	**HPLC**
1	78 ± 8	75 ± 11	147 ± 13	122 ± 24
2	159 ± 13	183 ± 4	219 ± 4	183 ± 4
3	235 ± 1	201 ± 4	96 ± 6	83.1 ± 0.4
4	288 ± 12	264 ± 4	132 ± 6	138 ± 18

*The humidity content of paprika powder was 8.5 ± 0.2 %.

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