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# Electrochemical ultra-micro sensors for the determination of synthetic and natural antioxidants in edible vegetable oils

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ABSTRACT

We describe the application of square wave voltammetry at ultramicroelectrodes for the determination of natural antioxidants (α, δ, and γ tocopherols), and tert-butyl hydroxytoluene in edible vegetable oils.

Tocopherol determinations were performed in benzene/ethanol (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$ + oil samples at a carbon fiber disk ultramicroelectrode, and tert-butyl hydroxytoluene was determined in acetonitrile (ACN) + 0.1 mol $L^{-1}$ $(C_4H_9)_4NF_6P$ at a Pt band ultramicroelectrode after performing its extraction from the oil sample with ACN.

Recovery percentages determined by the standard additions method were in the range from 92% to 102%, with variation coefficients between 0.5% and 4%.

Antioxidant concentrations calculated by this methodology were in good agreement with those values declared by the manufacturers.



## 1. Introduction

Seed oils are part of the human diet, and their production has increased in recent years due to the tendency of gradually replace animal fat by that of vegetable origin. Such changes arise from healthier lifestyles, which involve the consumption of foods rich in beneficial compounds for human health [1].

Some compounds such as polyphenols, flavonoids, and vitamin E have antioxidant activity and provide protection to cell membranes, preventing their oxidation by free radicals and their subsequent degradation, and provide protection against age-related diseases, cardiovascular disorders, or Alzheimer [2–4]. Considering that the human body does not synthesize these natural antioxidants, they must be incorporated through the diet. Some foods rich in these essential compounds are vegetable oils [3]. Vitamin E presents in vegetable oils is particularly important not only for its nutritional value but also because it helps to prevent oxidation of lipids, resulting in the formation of undesirable products which deteriorate oils [5–7]. Synthetic antioxidants have a similar function. Thus, tert-butyl hydroxyanisole (BHA), tert-butyl hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ), and propyl gallate (PG) are added to vegetable oils in amounts allowed by international law [8]. However, these synthetic antioxidants widely used in the food industry could be responsible for liver damage and carcinogenesis [9–12].

Vitamins E, A, D, and K are fat-soluble. Vitamin E consists of four tocopherols and four tocotrienols, which differ by the saturation of their side chains. Thus, tocopherols have a saturated chain and tocotrienols an unsaturated chain with three double bonds at carbons 3, 7 and 11 [13]. Within each group, the isomers differ in the number and position of methyl groups on the aromatic ring, and are called as α, β, γ and δ.

Various methods have been used for the determination of synthetic and natural antioxidants in edible oils [8], being the most used HPLC chromatography [13–17].

Moreover, studies have been conducted in recent years to the development of analytical techniques to determine the total content of tocopherols in vegetable oils as well as the differentiation and the determination of their isomers [3,18,19]. Electroanalytical techniques have also proved to be a convenient alternative to determine antioxidants in edible oils [20–24].

In this work, we propose a simple electroanalytical method to determine tocopherols, and BHT in edible vegetable oils based on

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the application of square wave voltammetry (SWV) at carbon fiber disk and Pt band ultramicroelectrodes (UME), and discuss results obtained by cyclic voltammetry (CV) at a conventional glassy carbon (GC) electrode.

## 2. Materials and methods

BHT, tocopherols, and $(C_4H_9)_4NF_6P$ (TBAHFP) were purchased from SIGMA Chemical Company, USA. Ethanol (EtOH), and $H_2SO_4$ were Merck p.a. Benzene (Bz), acetonitrile (ACN), and water were Sintorgan, HPLC grade. ACN was kept over 3 Å molecular sieves, and then used without further purification. Other reagents were used as received.

Edible oils were purchased in local supermarkets. Two reaction media were used for the quantification of antioxidants. Therefore, oils were dissolved in a mixture of Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$. This solution was then used to perform the determination of tocopherols. On the other hand, BHT was extracted with ACN from oils following a procedure previously described by us [24]. Then, its determination was carried out in ACN + 0.1 mol $L^{-1}$ TBAHFP.

A two-compartment pyrex cell using a conventional three-electrode configuration was used to perform SWV and CV experiments, which was coupled to an AutoLab PGSTAT 12 potentiostat, controlled by the GPES 4.9 electrochemical software. The characteristic parameters of SW voltammograms were square wave amplitude, $\Delta E_{SW} = 0.050$ V, staircase step height, $\Delta E_s = 0.005$ V, and frequency, $f = 25$ Hz. The scan rate ($v$) in CV was varied from 0.025 to 0.200 V $s^{-1}$.

Working electrodes were a carbon fiber disk UME (BAS Electroanalytical System, USA, diameter, $\emptyset = 11$ μm), a Pt band UME constructed in our laboratory as described in literature [25], and a GC disk (from BAS, $\emptyset = 3$ mm). The pretreatment of UME was previously described [20,26]. The GC electrode was polished with 0.3 and then 0.05 μm wet alumina powder (from Fischer), copiously rinsed with water, and sonicated in a water bath for 3 min. Then, the electrode was transferred to an electrochemical cell containing the corresponding supporting electrolyte, and cycled 10 times between 0 and 1 V. This pre-treatment produced an electrochemical activation of its surface and allowed to obtain reproducible responses. The reference electrode was an aqueous saturated calomel electrode (SCE), and the counter electrode was a Pt foil of large area ($A \approx 2$ $cm^2$).

All solutions were deoxygenated by bubbling $N_2$ prior to measurements. The standard additions method was used to determine recovery percentages from oil samples spiked with antioxidants. The temperature was 25.0 ± 0.5 °C.

## 3. Results and discussion

### 3.1. Qualitative determination in olive and corn oils

Fig. 1 shows cyclic voltammograms recorded for BHT in ACN + 0.1 mol $L^{-1}$ TBAHFP at a GC electrode at different v. An irreversible oxidation peak is clearly defined in the potential range from 0.95 to 1.45 V. Successive scans showed that voltammetric signals are highly reproducible. A plot of peak currents ($I_p$) as a function of $v^{1/2}$ was linear (inset Fig. 1), showing a diffusion control for the electrode process [27]. Similar results were found for the other antioxidants studied by CV (results not shown).

Then, we developed an electrochemical method for the determination of antioxidants in edible oils. We use an olive oil which has no synthetic antioxidants, and two corn oils, one of which contains BHT, and the other one does not contain any.

We first studied SWV responses in two reaction media using the corresponding commercial reagents. Thus, net peak potentials

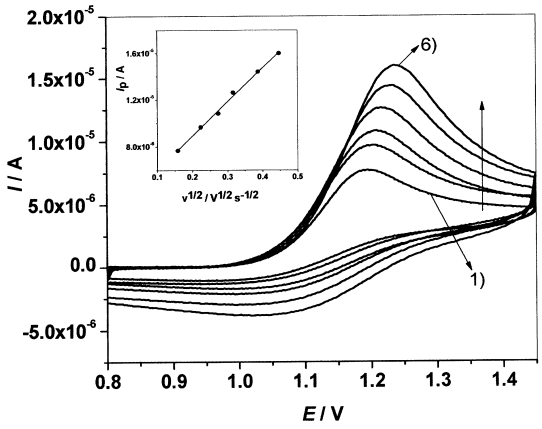


**Fig. 1.** Cyclic voltammograms recorded for BHT in ACN + 0.1 mol $L^{-1}$ TBAHFP at different scan rates. Working electrode: GC $c^*_{BHT} = 3.2 \times 10^{-4}$ mol $L^{-1}$. The scan rate was varied from 0.025 to 0.200 V $s^{-1}$ (1–6). Inset: plot of $I_p$ as a function of $v^{1/2}$.

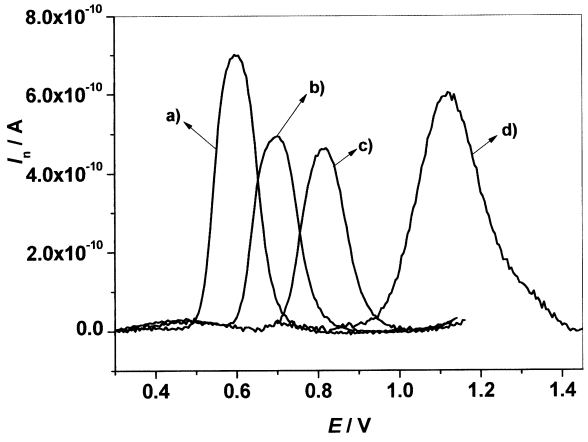


**Fig. 2.** Square wave voltammograms recorded for: (a) α, (b) γ, (c) δ tocopherols and (d) BHT at a CF disk UME ($\emptyset = 11$ μm) in Bz/EtOH (1:2) + 0.1 M $H_2SO_4$. Concentrations: $6.9 \times 10^{-4}$ M, $2.6 \times 10^{-4}$ M, $2.7 \times 10^{-4}$ M and $3.8 \times 10^{-4}$ M, respectively. $\Delta E_{SW} = 0.050$ V, $\Delta E_s = 0.005$ V, $f = 25$ Hz.

($E_{p,n}$) of α, γ, and δ tocopherols, and BHT were 0.59, 0.70, 0.81, and 1.1 V, respectively, in Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$ at a carbon fiber disk UME (Fig. 2). On the other hand, $E_{p,n}$ of α, γ, and δ tocopherols, and BHT in ACN + 0.1 mol $L^{-1}$ TBAHFP at a Pt band UME were 0.72, 0.82, 0.92, and 1.3 V, respectively (Fig. 3). We do

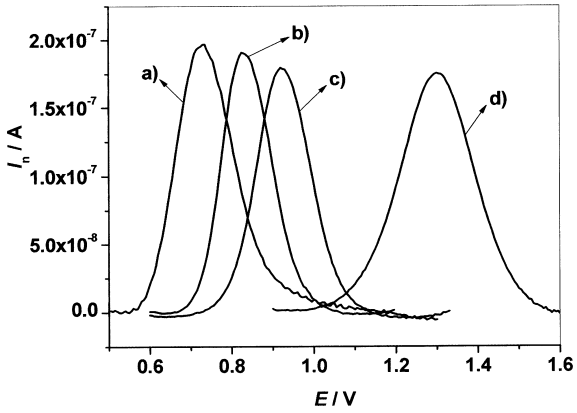


**Fig. 3.** Square wave voltammograms recorded for: (a) α, (b) γ, (c) δ tocopherols and (d) BHT at a Pt band UME in ACN + 0.1 M TBAHFP. Concentrations: $5.6 \times 10^{-5}$ M, $1.3 \times 10^{-4}$ M, $8.4 \times 10^{-5}$ M and $9.3 \times 10^{-5}$ M, respectively. Square wave parameters are the same as in Fig. 2.

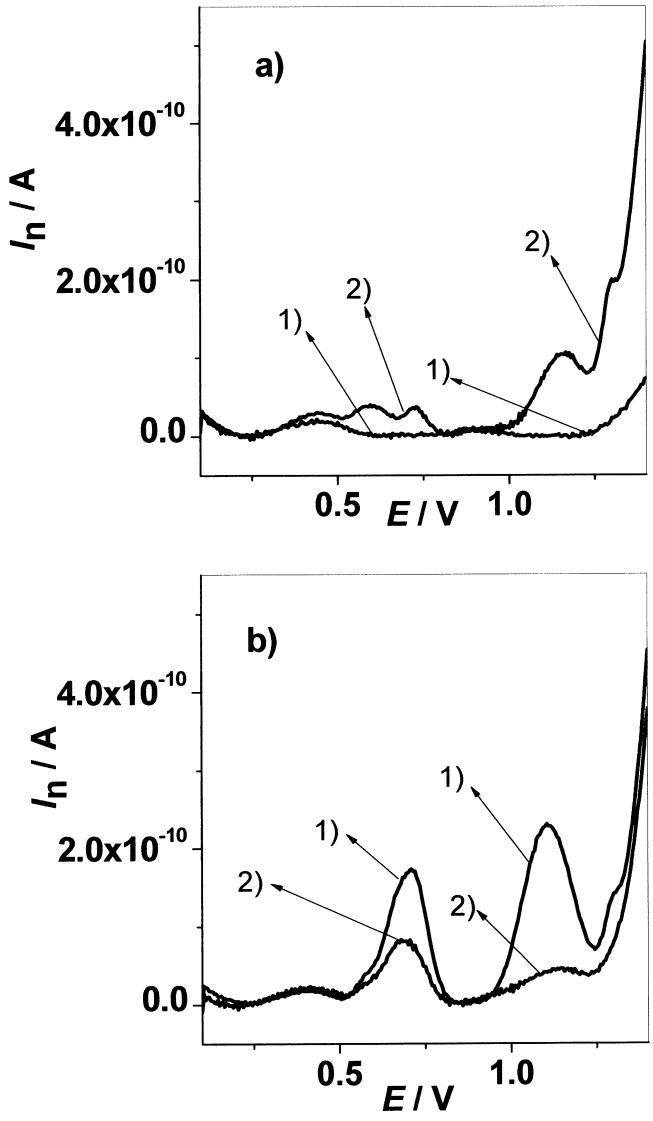


**Fig. 4.** (a) SW voltammograms recorded for Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$ (blank solution, line 1), and solution A (line 2). (b) SW voltammograms of an olive oil sample spiked with γ tocopherol (600 ppm), and BHT (200 ppm) before (line 1), and after (line 2) performing the extraction procedure with ACN. Working electrode: carbon fiber disk UME. Square wave parameters are the same as in Fig. 2. Solution A is composed by 1.5 mL of olive oil + 15 mL of Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$.

not study the β isomer because it is usually not present in vegetable oils analyzed or is present at ultra trace levels.

### 3.1.1. Olive oil

HPLC studies showed that the olive oil contains mainly α tocopherol, and different polyphenolic compounds [28], whereas γ and δ tocopherols are present at trace levels. Therefore, 1.5 mL of the oil was dissolved in 15 mL of Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$ (solution A), and transferred to the electrochemical cell. A SW voltammogram recorded at a carbon fiber disk UME showed four voltammetric peaks well defined, with $E_{p,n}$ at 0.59, 0.73, 1.16, and 1.3 V (line 2 in Fig. 4a). As it can be observed, the peak centered at about 0.41 V is also present in the blank solution (compare lines 1 and 2 in Fig. 4a). The peak centered at 0.59 V corresponds to α-tocopherol, and peaks at 0.73, 1.16, and 1.3 V would correspond probably to unidentified phenolic compounds present in the olive oil [28].

Then, the olive oil was spiked with $1.4 \times 10^{-3}$ mol $L^{-1}$ (600 ppm) and $9 \times 10^{-4}$ mol $L^{-1}$ (200 ppm) of γ-tocopherol and BHT, respectively. SW voltammograms recorded before and after performing the extraction procedure of antioxidants with ACN [24] are shown in Fig. 4b (lines 1 and 2, respectively). These voltammograms show two peaks well defined, with $E_{p,n}$ at 0.72 and 1.1 V, which can be assigned to the electrochemical oxidation of γ-tocopherol and BHT, respectively. These results indicate that γ-tocopherol has been extracted only in about 50% (compare lines 1 and 2 in Fig. 4b), whereas BHT is extracted in a very high percentage, as was previously discussed by us [24].

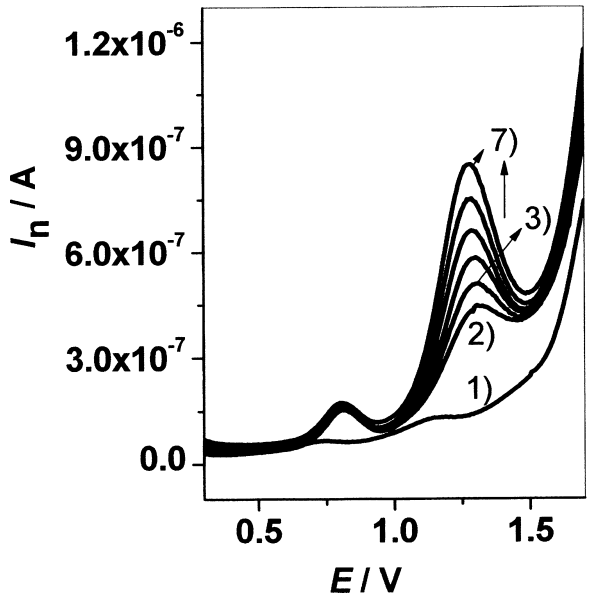


**Fig. 5.** SW voltammograms recorded for the extract of olive oil non-spiked with γ-tocopherol and BHT (line 1), for solution B (line 2), and for solution B after the addition of different BHT aliquots. BHT concentration was varied from $5.51 \times 10^{-5}$ (line 4) to $2.75 \times 10^{-4}$ mol $L^{-1}$ (line 8). Working electrode: Pt band UME. SWV parameters are the same that in Fig. 2. Solution B is that obtained from ACN extract of olive oil (previously spiked with 600 ppm of γ-tocopherol + 200 ppm of BHT), and dissolved in ACN + 0.1 mol $L^{-1}$ TBAHFP.

On the other hand, two portions of 3 mL of the olive oil were chosen. One of them was spiked with 600 ppm of γ-tocopherol, and 200 ppm of BHT. Then, both portions of the olive oil were subjected to a extraction procedure with ACN, and 0.1 mol $L^{-1}$ TBAHFP was added to each of the extracts (final volume = 10 mL). A blank of the extract of the portion non-spiked is shown in Fig. 5 (line 1). The SW voltammogram recorded at a Pt band UME in ACN + 0.1 mol $L^{-1}$ TBAHFP for the extract of spiked portion (solution B) shows two voltammetric peaks centered at $E_{p,n}$ = 0.80, and 1.30 V, which correspond to the electrochemical oxidation of γ-tocopherol and BHT, respectively (line 2 in Fig. 5). Then, several BHT aliquots were added to the electrochemical cell, and new SW voltammograms were recorded after each addition (lines 3–7 in Fig. 5). Thus, the peak centered at 1.3 V increases as the BHT concentration increases. Moreover, the peak centered at 0.8 V remains practically constant, which would evidence that any fouling phenomenon affects the working electrode surface, as often happens when the electroactive group is a phenolic species [29].

### 3.1.2. Corn oils

As it was previously described for the olive oil, we carried out the characterization of two corn oils, called "I" and "II", which contain α and δ-tocopherols at trace levels, and γ-tocopherol in a higher proportion. In addition, the oil "I" contains BHT, whereas the oil "II" does not contain this synthetic antioxidant, as stated by the manufacturer.

Therefore, 1.5 mL of both corn oils were dissolved in 15 mL of Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$ (solution C for oil "I", and

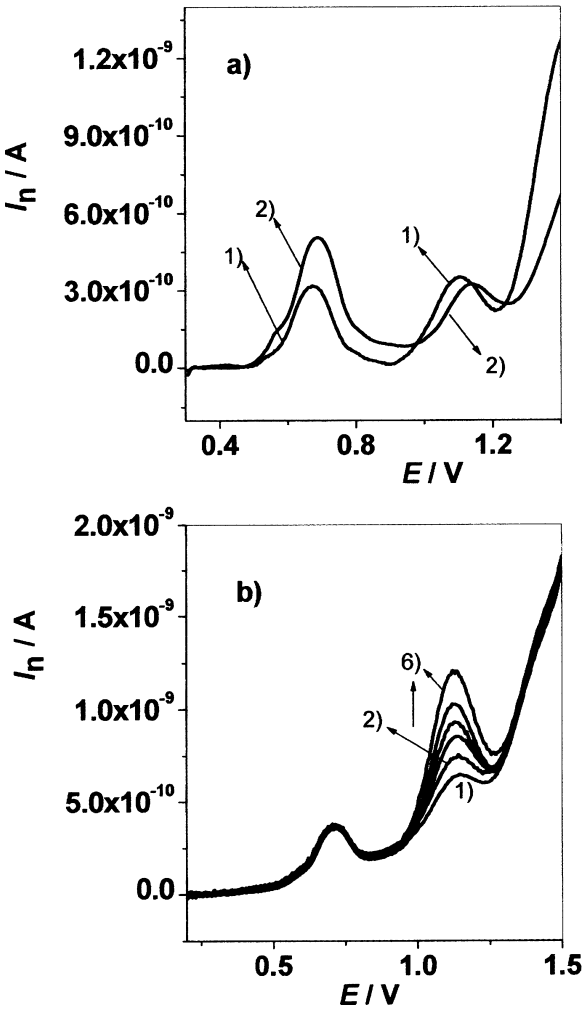


**Fig. 6.** (a) SW voltammograms recorded for solutions C (line 1), and D (line 2). (b) SW voltammograms recorded for solution C before (line 1), and after the addition of different BHT aliquots. BHT concentrations were in the range from $6.32 \times 10^{-5}$ mol $L^{-1}$ (line 2) to $3.78 \times 10^{-4}$ mol $L^{-1}$ (line 6). Working electrode: carbon fiber disk UME ($\varnothing$ = 11 μm). SWV parameters are the same that in Fig. 2. Solutions C and D were those obtained when 1.5 mL of corn oils "I" and "II" were dissolved in Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$.

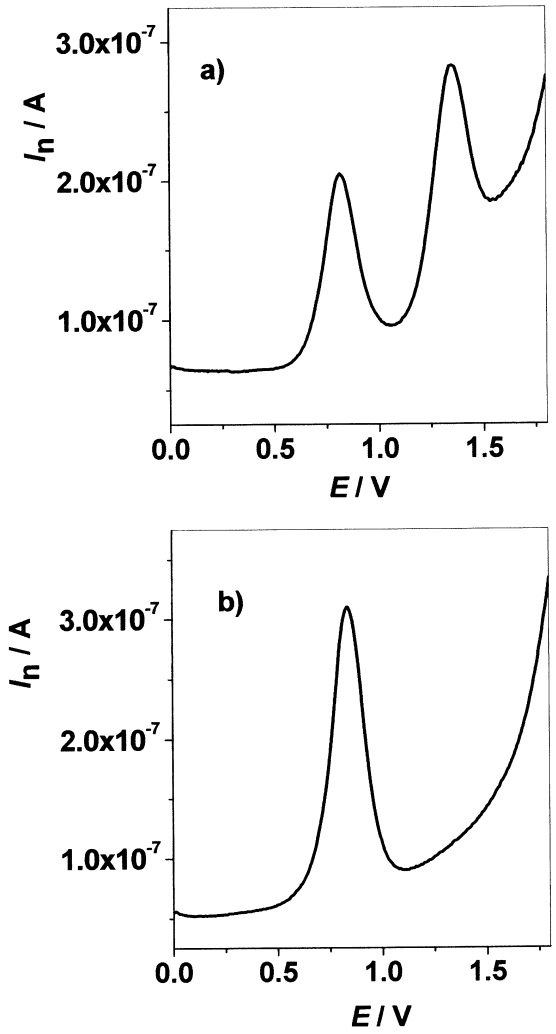


**Fig. 7.** SW voltammograms recorded for corn oil "I" (a), and corn oil "II" (b) after performing the extraction procedure with ACN. Reaction medium: ACN + 0.1 mol $L^{-1}$ TBAHFP. Working electrode: Pt band UME. SWV parameters are the same that in Fig. 2.

solution D for oil "II"), and SW voltammograms were recorded at a carbon fiber disk UME (Fig. 6a). Both voltammograms show peaks centered at about 0.7 V, which correspond to the electrochemical oxidation of γ-tocopherol, while solution C also shows a peak at 1.10 V (Fig. 6a, line 1), which corresponds to the oxidation of BHT [20], and solution D shows a peak at 1.15 V (Fig. 6a, line 2). Latter peak could be due to some compound present in the oil matrix that has not been identified yet.

Then, different BHT aliquots were added to the electrochemical cell, which contained the solution C (corn oil "I"). SW voltammograms were recorded after the addition of each aliquot (lines 2–6 in Fig. 4b). These results clearly show that the signal at 1.1 V corresponds to the electrochemical oxidation of BHT presents in the corn oil "I".

In addition, an extraction procedure with ACN was applied to both corn oils ("I" and "II"). SW voltammograms recorded in Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$ at a carbon fiber disk UME before and after performing the extraction procedure showed that the signal of γ-tocopherol decreases only about 40–50%, in good agreement with results previously found by us for the olive oil. Moreover, the signal at 1.10 V corresponding to the electrochemical oxidation of BHT decreased almost completely for the corn oil "I", while the signal at 1.15 V for the corn oil sample "II" remains practically constant, as expected (results no shown).

SW voltammograms of both corn oils recorded after performing antioxidants extraction with ACN are shown in Fig. 7a and b for oils "I" and "II", respectively. Both SW voltammograms show a peak at 0.80 V, which is due to the presence of γ-tocopherol in both corn oils, and which is only extracted in about 50%. The peak at 1.30 V (Fig. 7a), assigned to BHT oxidation, is only observed for the corn oil "I", in agreement with that specified by the manufacturer (BHT < 200 ppm).

Based on these results, the determination of tocopherols was performed in all cases in Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$ at a carbon fiber disk UME. On the other hand, the BHT determination was carried out in ACN + 0.1 mol $L^{-1}$ TBAHFP after performing the extraction procedure with ACN, considering that BHT is extracted in practically 100%, and interferences present in corn oils, which discharge at similar potentials that BHT, are not extracted by ACN.

### 3.2. Recovery assays

Recovery percentages for α, γ, and δ tocopherols were determined applying the standard additions method to solution A, which had previously been spiked with known amounts of antioxidants

**Table 1**
Recovery percentages obtained by the standard addictions method in olive oil samples spiked with tocopherols using SWV at a carbon fiber disk UME (ø = 11 μm). Reaction medium: Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$. Reference electrode: SCE. $\Delta E_{sw}$ = 0.050 V, $\Delta E_s$ = 0.005 V and $f$ = 25 Hz.

| Samples | Tocopherols | $10^{12}$ slope[a]/A ppm$^{-1}$ | $10^{11}$ intercept[b]/A | $r$[c] | Added $c^*_{Aox}$/ppm | $10^{-2}$ calculated[d] $c^*_{AoX}$ | Recovery % | % RSD[e] |
|---|---|---|---|---|---|---|---|---|
| 1 | | 2.80 ± 0.05 | 3.5 ± 0.2 | 0.9805 | 150 | 1.38 ± 0.08 | 92.0 | |
| 2 | Delta | 3.15 ± 0.06 | 8.1 ± 0.1 | 0.9890 | 300 | 2.83 ± 0.02 | 9.3 | 2.1 |
| 3 | | 3.22 ± 0.04 | 19.9 ± 0.1 | 0.9815 | 600 | 5.77 ± 0.01 | 96.2 | |
| 4 | | 3.53 ± 0.04 | 11.9 ± 0.1 | 0.9998 | 400 | 3.70 ± 0.09 | 92.5 | |
| 5 | Gamma | 4.15 ± 0.03 | 21.5 ± 0.1 | 0.9816 | 600 | 5.7 ± 0.2 | 95.0 | 2.5 |
| 6 | | 4.32 ± 0.02 | 30.5 ± 0.1 | 0.9843 | 800 | 7.8 ± 0.3 | 97.5 | |
| 10 | | 2.15 ± 0.08 | 2.85 ± 0.07 | 0.9898 | 150 | 1.46 ± 0.05 | 97.3 | |
| 11 | | 1.93 ± 0.02 | 1.49 ± 0.01 | 0.9801 | 90 | 0.85 ± 0.03 | 94.4 | |
| 12 | Alpha | 2.19 ± 0.06 | 4.14 ± 0.3 | 0.9941 | 215 | 2.08 ± 0.05 | 96.7 | 2.9 |
| 13 | | 2.24 ± 0.05 | 4.3 ± 0.3 | 0.9967 | 215 | 2.1 ± 0.1 | 97.7 | |
| 14 | | 2.21 ± 0.06 | 4.5 ± 0.1 | 0.9961 | 215 | 2.2 ± 0.1 | 102.3 | |

[a, b, c] are slope, intercept, and linear regression coefficient, respectively, of the $I_{p,n}$ (A) vs $c^*_{Aox}$ (ppm) plot.
[d] determined taking into account the dilutions carried out.
[e] is the percent relative standard deviation of the percent recovery obtained from three different additions of the antioxidant, $c^*_{Aox}$.

(Table 1). We used three different initial concentration levels for each antioxidant (Table 1). Therefore, SW voltammgrams obtained after the addition of each antioxidant aliquot were subtracted by the corresponding blank of the oil matrix (line 2 in Fig. 4a). Net peak currents ($I_{p,n}$) were plotted as a function of the antioxidant bulk concentration ($c^*$). $I_{p,n}$ vs $c^*$ plots were linear in the concentration range from $3.4 \times 10^{-5}$ to $2.2 \times 10^{-4}$ mol $L^{-1}$, $2.7 \times 10^{-5}$ to $1.6 \times 10^{-4}$ mol $L^{-1}$, and $3.1 \times 10^{-5}$ to $2.0 \times 10^{-4}$ mol $L^{-1}$ for α,

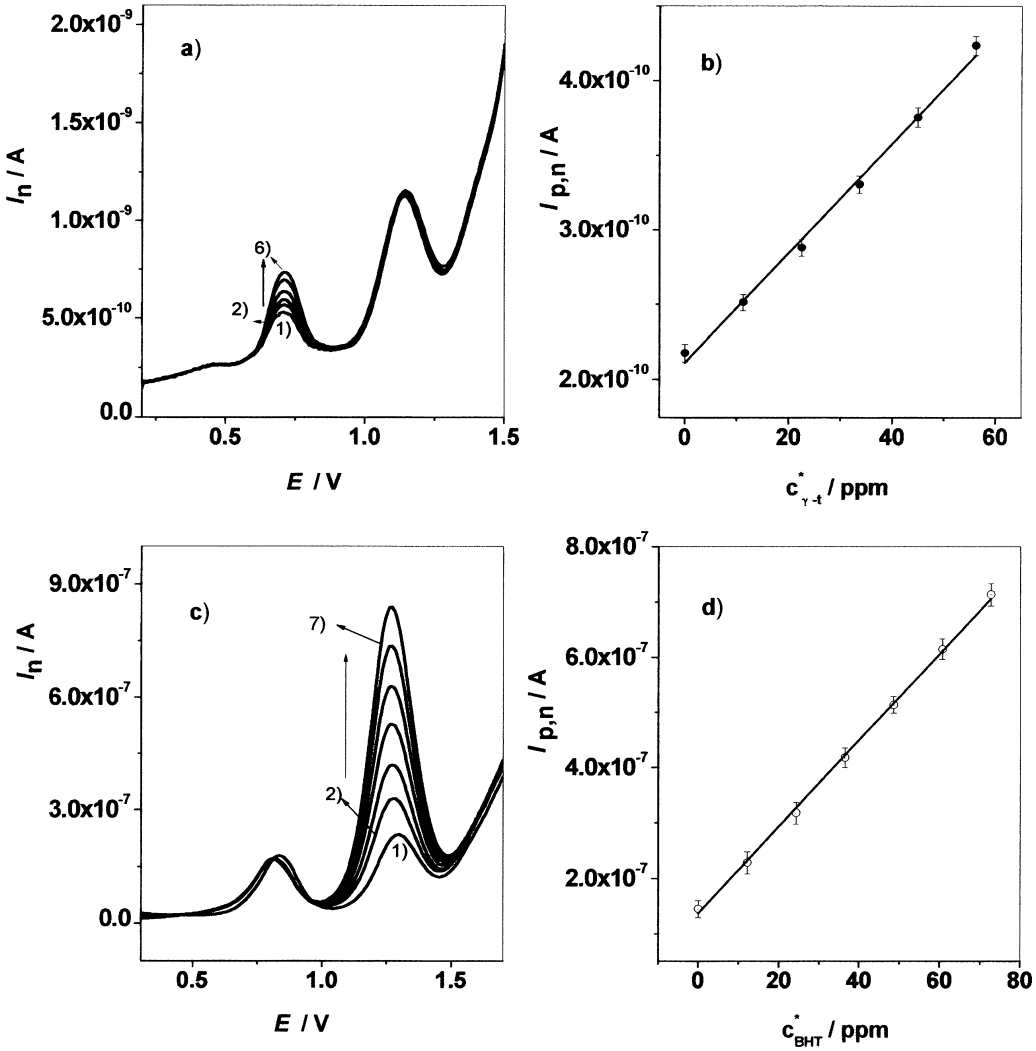


**Fig. 8.** (a) SW voltammograms recorded for solution C in Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$ before (line 1), and after the addition of different γ-tocopherol aliquots, whose concentration was varied from $2.70 \times 10^{-5}$ (line 2) to $1.35 \times 10^{-4}$ mol $L^{-1}$ (line 6). (b) Plot of $I_{p,n}$ as a function of $c^*_{\gamma-t}$. Working electrode: carbon fiber disk UME (ø = 11 μm). (c) SW voltammograms recorded for solution C in ACN + 0.1 mol $L^{-1}$ TBAHFP after performing the extraction procedure with ACN (line 1), and those recorded after the addition of different BHT aliquots, whose concentration was varied from $5.51 \times 10^{-5}$ (line 2) to $3.30 \times 10^{-4}$ mol $L^{-1}$ (line 7). (d) Plot of $I_{p,n}$ as a function of $c^*_{BHT}$. Working electrode: Pt band UME. SWV parameters are the same that in Fig. 2.

**Table 2**
Antioxidant contents in different edible oils, and parameters obtained from the standard additions method by SWV at a carbon fiber disk UME ($\emptyset$ = 11 μm) in Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$ for natural antioxidants, and at a Pt band UME in ACN + 0.1 mol $L^{-1}$ TBAHFP for the synthetic antioxidant. $\Delta E_{sw}$ = 0.050 V, $\Delta E_s$ = 0.005 V, and $f$ = 25 Hz. Reference electrode: SCE.

| Oil samples | Antioxidants | $10^{12}$ slope[a]/A $ppm^{-1}$ | $10^{11}$ intercept[b]/A | r[c] | $10^{12}$ residual standard deviation/A | LOD/ppm | LOQ/ppm | $10^{-2}$ calculated[d] $c^*_{AoX}$ | Vitamin E/ppm (amount declared by the manufacturer) |
|---|---|---|---|---|---|---|---|---|---|
| Corn "I" | γ | 3.7 ± 0.1 | 2.11 ± 0.04 | 0.9945 | 5.7 | 6.2 | 14.4 | 6.3 ± 0.1 | 590 |
| Corn "II" | γ | 4.4 ± 0.1 | 4.5 ± 0.1 | 0.9988 | 17 | 14.2 | 35.2 | 7.8 ± 0.2 | 760 |
| Olive | α | 2.15 ± 0.04 | 4.2 ± 0.2 | 0.9978 | 3.0 | 5.1 | 12.8 | 2.20 ± 0.09 | 217 |
| Sunflower | α | 2.81 ± 0.06 | 1.06 ± 0.05 | 0.9975 | 7.8 | 10.7 | 25 | 4.2 ± 0.1 | 438 |
| Soybean | γ | 3.65 ± 0.04 | 2.79 ± 0.02 | 0.9993 | 2.4 | 2.4 | 5.95 | 8.40 ± 0.04 | 898 |
| Canola | γ | 4.02 ± 0.08 | 2.17 ± 0.04 | 0.9980 | 5.7 | 5.7 | 13.4 | 5.9 ± 0.09 | 650 |
| Grape seeds | α | 2.62 ± 0.09 | 8.4 ± 0.4 | 0.9944 | 5.4 | 7.8 | 18.2 | 3.5 ± 0.1 | 335 |
| Corn "I" | BHT | 7900 ± 100 | 13500 ± 500 | 0.9988 | 7200 | 3.3 | 8.1 | 1.43 ± 0.01 | <200 |

[a, b, c] are slope, intercept and linear coefficient regression, respectively, of $I_{p,n}$ as a function of $c^*_{Aox}$ (ppm).
[d] Calculated taking into account the dilution effects.

γ, and δ tocopherols, respectively. Standard deviations, recovery percentages (calculated taking into account dilutions performed), and percentage relative standard deviations (% RSD) obtained from linear regression parameters [30] are shown in Table 1. The reproducibility of the method was determined using three concentration levels, and performing measurements in triplicate for each solution (three new solutions, and three new electrodes were used). % RSD values were in the range from 2 to 3%, as shown in Table 1. The repeatability was determined on the recoveries obtained for α-tocopherol solutions, measuring three identical addition levels (each in triplicate), and using the same carbon fiber disk UME. Thus, the %RSD was about 3%. The recovery percentages were very good for all antioxidants, i.e., they were 92–98% for tocopherols, and 102% for BHT.

### 3.3. Qualitative and quantitative analysis of commercial oil samples

Edible vegetable oils analyzed by the standard additions method were those of the most common varieties of the southern of the province of Córdoba, Argentina, such as sunflower, corn, soybean, and olive oils, and two alternative varieties like canola and grape seeds oils. In these varieties is usual to find one of the isomer of vitamin E in a higher proportion, and traces of others. However, soybean and sunflower oils usually have a similar proportion of two isomers, γ + δ, and α + γ, respectively. Thus, we express natural antioxidants as equivalents of tocopherol which is present in a higher proportion in oil, obtaining the total content of tocopherols, usually equivalent to what declared as vitamin E by the manufacturer. We also determined the presence or absence of BHT in different oil samples, verifying that it does not exceed the maximum level allowed by the Código Alimentario Argentino [31].

We show as an example SW voltammograms obtained for the corn oil "I", according to the methodology proposed (Fig. 8). Results obtained for other oils are summarized in Table 2. SW voltammograms recorded after performing the addition of different γ-tocopherol aliquots to a solution of the corn oil "I" dissolved in Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$ are shown in Fig. 8a. The signal centered at about 0.70 V increases as the γ-tocopherol concentration increases, and the signal at about 1.15 V (BHT) keeps practically unchanged. A plot of the $I_{p,n}$ as a function of the γ-tocopherol bulk concentration, $c^*_{\gamma-t}$ (expressed as ppm), is shown in Fig. 8b. The slope, the intercept, and respective parameters which characterize the quality of results (the residual standard deviation, limit of detection (LOD), and limit of quantification (LOQ)) were determined from the linear regression parameters [30,32]. Therefore, the γ-tocopherol content was equal to $(6.3 \pm 0.1) \times 10^2$ ppm, with a variation coefficient of about 1.6% (Table 2).

SW voltammograms recorded for the corn oil "I" in ACN + 0.1 M TBAHFP, after performing the extraction procedure with CAN, are shown in Fig. 8c. The signal at 0.80 V corresponds to the γ-tocopherol, which is partially extracted (about 50%), and the signal at 1.30 V corresponds to the BHT, which is extracted almost 100%. The effect on peak currents for different BHT aliquots added to the electrochemical cell is also shown in Fig. 8c. A plot of $I_{p,n}$ as a function of BHT bulk concentration, $c^*_{BHT}$, is shown in Fig. 8d. The BHT concentration calculated for the corn oil "I" was (143.5 ± 0.8) ppm, with a variation coefficient of 0.5%, not exceeding the maximum level allowed by the law (200 ppm) [31].

The content of natural and synthetic antioxidants in different oils is shown in Table 2. As expected, the presence of BHT was only detected in the corn oil "I". Satisfactory LOD and LOQ were obtained for all antioxidants. Particularly, the LOD calculated for BHT is well below the maximum permitted by actual regulation [32]. In addition, values obtained for the different antioxidants are compared with those informed in labels by the manufacturers. The differences between values calculated and those reported by the manufacturer were 6.8, 2.6, 1.4, 4.1, 6.5, 9.2 and 4.5% for corn "I", corn "II", olive, sunflower, soybean, canola and grape seeds, respectively (see Table 2).

## 4. Conclusions

The determination of tocopherols in vegetable edible oils was performed in Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$, whereas the BHT determination was carried out in ACN + 0.1 mol $L^{-1}$ TBAHFP after performing the extraction procedure of the antioxidant with acetonitrile.

Recovery percentages were very good for all antioxidants. They were in the 92–98% range for tocopherols whereas recovery for BHT was 102%. Percentage relative standard deviations for reproducibility and repeatability were below 3%. Variation coefficients were below 3% in the most of tocopherol measurements in Bz/EtOH (1:2) + 0.1 mol $L^{-1}$ $H_2SO_4$, while the variation coefficient was 0.5% for the BHT determination in ACN + 0.1 mol $L^{-1}$ TBAHFP. Limits of detection and quantification were satisfactory. Results obtained show clearly that this electroanalytical methodology is useful for the determination of natural and synthetic antioxidants in edible vegetable oils.

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