Synthetic Antioxidants in Edible Oils by Square-Wave Voltammetry on Ultramicroelectrodes

Claudio Ceballos[a] **and Héctor Fernández**[b,*]

Departamento de [a]Ciencias Básicas y Departamento de Tecnología Química-Facultad de Ingeniería,
and de [b]Química y Física-Facultad de Ciencias Exactas, Fisico-Químicas y Naturales, Universidad
Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina

ABSTRACT: Square wave voltammetry (SWV) on carbon disk ultramicroelectrodes (UME) was used to determine antioxidants *tert*-butylhydroxyanisole (BHA) and *tert*-butylhydroxytoluene (BHT) in vegetable oils. Direct determinations were accomplished in benzene/ethanol/H_2SO_4 solutions or in acetonitrile (ACN) after an extractive procedure. Much better recovery percentages were obtained in ACN extracts. By comparing with high-performance liquid chromatography (HPLC) results, BHA has a slightly lower recovery percentage by the SWV technique on ACN extracts. On the other hand, BHT shows a greater recovery percentage using the methodology proposed here. The analysis time through SWV is less than an hour for a duplicated analysis while the HPLC technique needs a greater time, besides the overnight stored time required by the current methodology. The present work tends to show that SWV on carbon disk UME allows sensitive, reproducible, and faster determination of BHA, BHT, or antioxidant mixtures in oil-solvent solutions or after a simple extractive procedure with the advantage of a higher recovery percentage. This validates the methodology as an analytical alternative for antioxidant quantification.

Paper no. J9364 in *JAOCS 77,* 731–735 (July 2000).

KEY WORDS: Antioxidant electroanalytical determination, BHA, BHT, carbon disk ultramicroelectrode, nonaqueous solvents, square-wave voltammetry, standard addition method, synthetic antioxidants, vegetable oils.

During recent years, many articles claim the advantage of the use of electrochemistry in food process or analysis (1–5). In the analytical field, electrochemical techniques were used to determine and quantify phenolic compounds used as antioxidants (6–10). *tert*-Butylhydroxyanisole (BHA) and/or *tert*-butylhydroxytoluene (BHT) are the most common antioxidants used in the lipid industry to improve stability and prevent rancidity in their products (11–13). Thus, the identification and quantification of antioxidants play an important role in the quality assignment of lipid compounds.

Both the AOAC (14) and AOCS Official Methods and Recommended Practices (15) include the determination of several antioxidants with acetonitrile (ACN) extraction step followed by high-performance liquid chromatography (HPLC) separation with ultraviolet (UV) detection at 280 nm.

Electrochemistry on ultramicroelectrodes (UME) appears as a good alternative for electroanalysis, particularly for the quantification of synthetic antioxidants (16–21).

On the other hand, pulse voltammetric techniques, especially square-wave voltammetry (SWV), have been proposed as alternative methods to decrease the time needed for analytical purposes (7,22). Thus, it is also possible to combine disk UME with SWV (23) to obtain highly reproducible signals, to minimize the cleaning difficulties of the UME and to improve both speed and sensitivity compared with other voltammetric methods (7,18,24).

In this work, direct measurements of antioxidants on vegetable oil solutions as well as determinations after extraction steps followed by SWV detection are studied, and both are compared with the results obtained by HPLC methods.

EXPERIMENTAL PROCEDURES

Instrumentation and methods. Measurements were performed in a two-compartment Pyrex cell. SWV experiments were done under a three-electrode configuration. An EG&G PAR 273 Potentiostat/Galvanostat with PAR 270 software were used. All square-wave voltammograms were obtained by using a frequency of 25 Hz, a pulse height (Esw) of 50 mV, and a potential step increment (ΔEsc) of 5 mV.

The standard addition method (25) was used when vegetable oil was present in the solution as well as in solutions after extraction steps with ACN.

Electrodes and material. The working electrode was a carbon fiber disk UME of about 33 µm diameter constructed in our laboratory. It was pretreated as explained elsewhere (19,20).

An aqueous saturated calomel electrode (SCE) and a silver wire (Alfa, 99.99%) were used as reference and quasi-refer-

---

*To whom correspondence should be addressed at Departamento de Química y Física-Facultad de Ciencias Exactas, Fisico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Agencia Postal No. 3 (5800), Rio Cuarto, Córdoba, Argentina. E-mail: HFERNANDEZ@exa.unrc.edu.ar

ence electrodes, respectively. The counter electrode was a platinum foil of large area (*ca*. 2 cm^2).

Analytical-grade BHA and BHT (Sigma Chemical Co., St. Louis, MO) were used as received. ACN was Sintorgan HPLC-grade. Benzene (Bz) and ethanol (EtOH) were Sintorgan UV-Visible and Merck p.a. (Darmstadt, Germany), respectively. The solvents were kept over 3 or 4 Å molecular sieves for 48 h prior to use and were then used without further purification.

Sulfuric acid (Merck p.a.) was used as received as supporting electrolyte.

HPLC measurements were performed under slightly different conditions than those proposed in the literature (14,15, 26,27). Experimental conditions were: C-18 reverse-phase column. Mobile phase: (i) 5% acetic acid aqueous solution, (ii) (1:1) ACN/methanol; from 30% of (ii) in (i) to 100% of (ii). Flow rate: 0.5 mL/min. A 10 µL sample loop injection valve was used and λ was 280 nm. The chromatograph was a Varian 5020 HPLC with UV-visible detector.

Commercial corn oil with no antioxidant added (according to the manufacturer label) was obtained from a local supermarket. The edible oil used was one widely consumed in Argentina. The official methods as well as the electrochemical one here proposed were used to verify the absence or presence of synthetic antioxidants. When necessary, binary solvent mixtures were used to dissolve the oil for the direct determination of the antioxidant(s) added in our laboratory to the actual samples. Electroactive reagent (antioxidant) concentrations were in the range from the experimental detection limit up to about 550 ppm.

Preparation of solutions. (i) Oil standard solution. Oil solutions spiked with BHA and BHT were prepared by adding appropriate aliquots of each antioxidant in isopropanol to tared flasks containing degassed vegetable oil without antioxidants. Isopropanol was removed under vacuum with stirring and gentle heat. The spiking levels were prepared between 50 and 200 ppm to evaluate the voltammetric performance from low concentrations up to about the higher limits level permitted by the Código Alimentario Argentino (28).

(ii) Oil solution in Bz/EtOH/H^+. The oil solutions used for voltammetric determination were prepared by dissolution of 1.5 g of oil in 15 mL of Bz/EtOH/H^+ (sulfuric acid) (1:2:0.1 M) solution.

ACN extractive procedure. A thoroughly extractive procedure with ACN in edible oils supplemented with BHA and BHT consisted of adding about 5 mL of ACN to 3 g of oil, sonicating for 4 min, and then transferring the supernatant extract to a 25-mL volumetric flask. This procedure was repeated four times, diluting with ACN. SWV techniques were used on these solutions. Extractive procedure used in the HPLC determinations was performed following the methodology recommended by the AOAC or AOCS (14,15).

RESULTS AND DISCUSSION

SWV on the oil/acid/solvent mixture. Corn oils or oil mixtures with corn oil as one component were used to evaluate substances that may interfere with antioxidant determination, particularly at high potential. Highly reproducible square-wave voltammograms of commercial corn oil without synthetic antioxidant, according to the manufacturer label, were obtained under different experimental conditions on a carbon disk UME in a (1:2:0.1 M) Bz/EtOH/H^+ solvent mixture. An oxidation peak corresponding to tocopherols (natural antioxidants) appears at about 0.65 V vs. SCE, and a shoulder of unknown nature is obtained at about 1.25 V vs. SCE in agreement with results obtained previously (6,7,19,20). Peak potentials for the discharge of both antioxidants are about 0.80 and 1.20 V vs. SCE, respectively.

The square-wave voltammogram of the solvent mixture with no oil added as well as in the presence of antioxidants shows no current signal at the 1.25 V potential region. The results found with oil added to the solution show that during the oxidation scan the shoulder at 1.25 V in a (1:2:0.1 M) Bz/EtOH/H^+ solvent mixture corresponds to an interference which belongs to the corn oil matrix. It does not have any assignment yet and appears in the same oxidation potential region of BHT. BHT was discarded as responsible for that shoulder on account of the results obtained through HPLC (9,10), i.e., no BHT signal was obtained after injection of the sample.

SWV on the ACN extract. Extraction with ACN was used to evaluate the performance of the electrochemical technique and to verify the possible presence of BHT in commercial corn oil.

Figure 1 shows the voltammograms obtained after the extraction procedure on commercial oil with different standard additions of BHA and BHT. From the voltammogram with no added antioxidants, clearly BHT or another synthetic antioxidant is absent from the oil, in agreement with the results of HPLC. Only the peak for tocopherols is present, indicating that they are also extracted by ACN from the oil. Three voltammetric runs were performed for each standard addition and, as is evident from Figure 1, the repeatability shown from the voltammograms obtained is excellent.

Previous results using cyclic voltammetry on carbon disk UME show better calibration curves and higher slope values in extracted antioxidants systems than those obtained in Bz/EtOH/H^+ solutions (20). The baseline in cyclic voltammograms is modified by the presence of the lipid matrix, and it is not the same as that obtained in no oil added to the solutions. The importance of this effect increases as the oxidation potential does. Then, the detection limit and sensitivity from the BHT calibration curve are affected (20). Therefore, the SWV methodology already tested for identification purposes (see above) was also tested in oil/solvent solutions and in solutions of antioxidants/ACN prepared from extracts to determine the concentration values of antioxidants.

We found no significant difference in square-wave voltammograms immediately recorded after an extraction procedure using ACN or even in a solution stored overnight. Therefore, SWV technique shows good reproducibility of data and is considerably faster than HPLC methods.

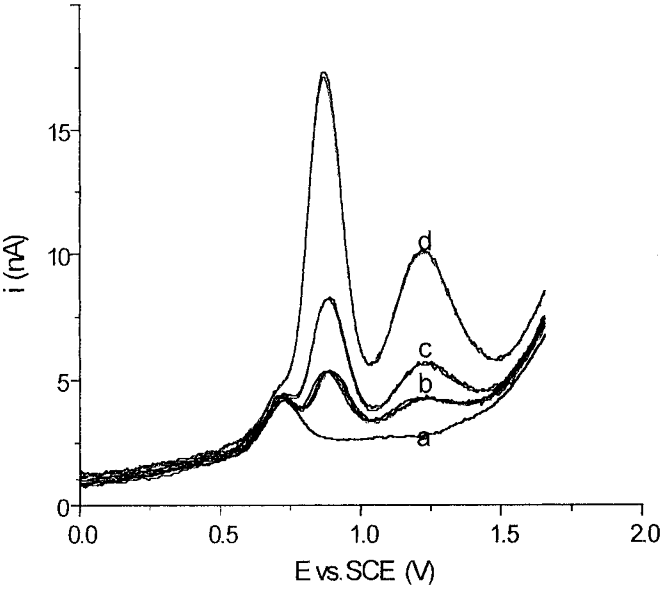

FIG. 1. Square-wave voltammograms obtained in corn oil extracts in 0.1M H_2SO_4/acetonitrile (ACN) solution after extraction. Working electrode: ultramicroelectrodes (UME) of carbon disk; reference electrode: saturated calomel electrode (SCE). $f = 25$Hz; $\Delta E_{SC} = 5$ mV; $E_{SW} = 50$ mV. (a) Extract with no added synthetic antioxidants; (b) three anodic potential sweeps on extract with added 68.33 ppm of *tert*-butylhydroxyanisole (BHA) and 69.06 ppm of *tert*-butylhydroxytoluene (BHT); (c) idem (b) but with 136.66 ppm of BHA and 138.12 ppm of BHT; (d) idem (c) but with 345 ppm of BHA and 341.65 ppm of BHT. i(nA), current (nanoamperes).

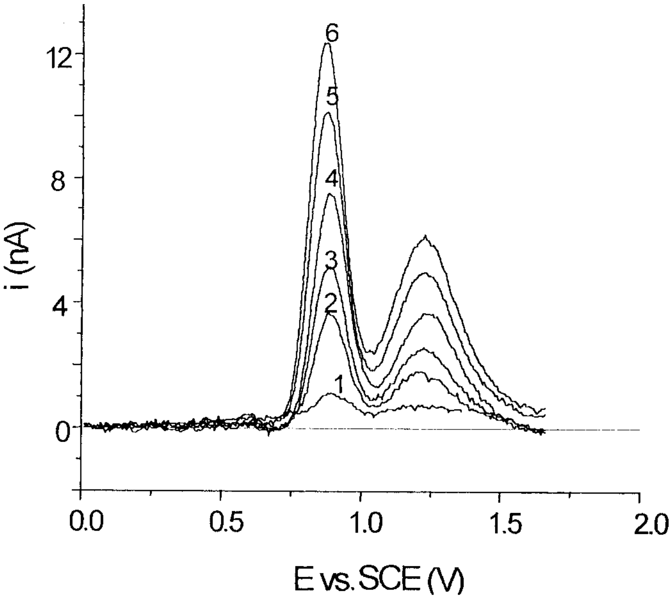

FIG. 2. Square-wave voltammograms obtained in corn oil extracts in 0.1M H_2SO_4/ACN solution after extraction. Conditions as those of Figure 1. "a" curve of Figure 1 was subtracted from all curves. (1) Extract of oil sample with previous addition of 51.80 ppm of BHA and 51.25 ppm of BHT; (2), (3), (4), (5), and (6) same as (1) but with added: 120.86, 189.92, 258.98, 328.04, and 397.10 ppm of BHA and 119.58, 187.91, 256.24, 324.57, and 392.90 ppm of BHT, respectively. See Figure 1 for abbreviations.

The standard addition method was used to quantify BHA and BHT on the ACN solution after an extraction procedure.

Figure 2 shows a set of square-wave voltammograms of antioxidants extracted under different conditions. The "a" curve of Figure 1 was subtracted from all curves. Voltammogram 1 is obtained from an extract (M1) of an oil sample which was spiked with 51.80 ppm of BHA and 51.25 ppm of BHT. Voltammograms were obtained, from this extract, after standard addition of antioxidants indicated in Figure 2 legend, i.e., M1 was subjected to the standard addition mentioned. Another set of voltammograms (not shown) was also obtained starting from extract M2 obtained from another oil sample spiked with 207.20 ppm BHA and 205.00 ppm BHT. In the same way as with M1, five consecutive additions of 69.06 ppm each were accomplished on M2 extract.

The maximal current of each peak in these voltammograms was measured and plotted against the concentration of antioxidants in the mass of oil used. This procedure was used to quantify the antioxidants by the standard addition method using a linear least square regression, for both initial concentrations, extracts M1 and M2, and in different media through direct and from extracts measurements. In all cases, linear calibration curves were obtained with a correlation coefficient higher than 0.997 using a confidence level of 95% according to the student's *t*-test.

Reproducibility of results and recovery percentage in Bz/EtOH/H$^+$ *mixtures.* Table 1 shows the results over 10 determinations (n) for each of the added antioxidant–oil/Bz/EtOH/H$^+$ solutions starting from different concentrations of antioxidants. Statistical criteria were used to evaluate the reproducibility relative standard deviation (RSD_R) and the recovery percentage (%R) over all determinations performed. Results show that in an oil–Bz/EtOH/H$^+$ solution with lower concentrations of antioxidants high values of RSD_R are obtained. Also, a low recovery percentage was obtained for BHT in oil–Bz/EtOH/H$^+$ solutions.

Better RSD_R are observed (10–13%) for the higher concentrations of antioxidants, but recovery percentages are lower than those reported from a collaborative study using the HPLC official method (99.4% BHA; 84.0% BHT) (26,27).

SWV analysis of antioxidants extracted using ACN. The use of an extractive procedure increases the analysis time as

TABLE 1
BHA and BHT Results in Oil–Bz/EtOH/H$^+$ Solution

Antioxidant	$C_{AO}{}^a$ (ppm)	n	$\bar{x}^b$ (ppm)	RSD_R (%)	R (%)
BHA	51.80	10	41.08	23.3	79.30
BHT	51.25	10	24.26	37.3	47.34
BHA	207.2	10	135.28	13.3	65.29
BHT	205.0	10	129.68	10.4	63.26

[a] C_{AO}, antioxidant concentration.
[b] $\bar{x}$, Average of antioxidant concentration. BHA, *tert*-butylhydroxyanisole; BHT, *tert*-butylhydroxytoluene; Bz/EtOH/H$^+$, benzene/ethanol/sulfuric acid; RSD_R, relative standard deviatoin; R (%), recovery percentage.

compared with direct measurements in Bz/EtOH/H^+ medium although the standard deviation decreases and greater recovery percentages are obtained. Figure 3 shows a representative determination of BHT by the standard addition method, with a recovery percentage of 94.4% and a RSD_R of 2.3%.

Square-wave voltammograms of an antioxidant extracted in ACN solution obtained on carbon disk UME with aliquots of BHT at fixed BHA concentration and then, at a maximal fixed concentration of BHT, with successive additions of BHA, are shown in Figure 4. At high concentrations, BHA interferes over the BHT peak although with an adequate selection of baseline, even with a higher error, tentative determination of BHT could still be performed under this condition.

Figure 5 shows the equivalence between the results of the square-wave method and the HPLC determinations obtained over the same analyzed solutions. HPLC determinations were performed under the conditions indicated in the Experimental Procedures section. As it can be observed, equivalence between both methods applied on three particular samples is clearly good. On the other hand, average recovery values together with statistical results for BHA and BHT obtained over a number of 10 samples, respectively, are depicted in Table 2. Average recovery values from a collaborative study (27) for the same antioxidants using a HPLC technique are also shown in Table 2. By comparing with the HPLC results, BHA has a slightly lower recovery percentage by the SWV technique on ACN extracts. On the other hand, BHT shows a greater recovery percentage using the methodology here proposed. Standard deviations from determinations are quite similar for both methods.

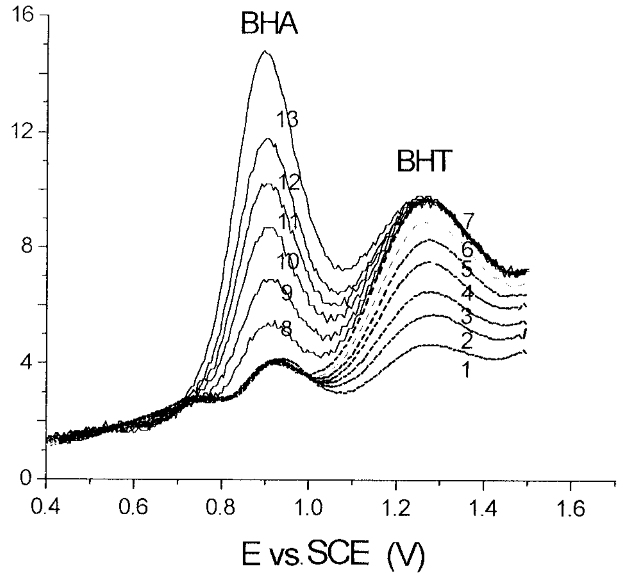

FIG. 4. Square-wave voltammograms of BHA and BHT obtained in extracts of oil sample/0.1 M H_2SO_4/ACN. Successive additions of BHT (1–7) at a fixed low value of BHA (1) and then, at a fixed value of BHT (7), successive additions of BHA (8–13). C_{BHT} from (1) to (7): 62.2, 124.4, 186.6, 248.8, 311.0, 373.2, and 435.4 ppm, respectively. C_{BHA}: for (1) and from (8) to (13): 60.4, 120.8, 181.2, 241.6, 302.0, 362.4, and 422.8 ppm, respectively. Parameters of SWV same as in Figure 1. See Figures 1 and 3 for abbreviations.

The analysis time through SWV is less than an hour for a duplicated analysis while the HPLC technique needs greater time, besides the overnight stored time required by the cur-

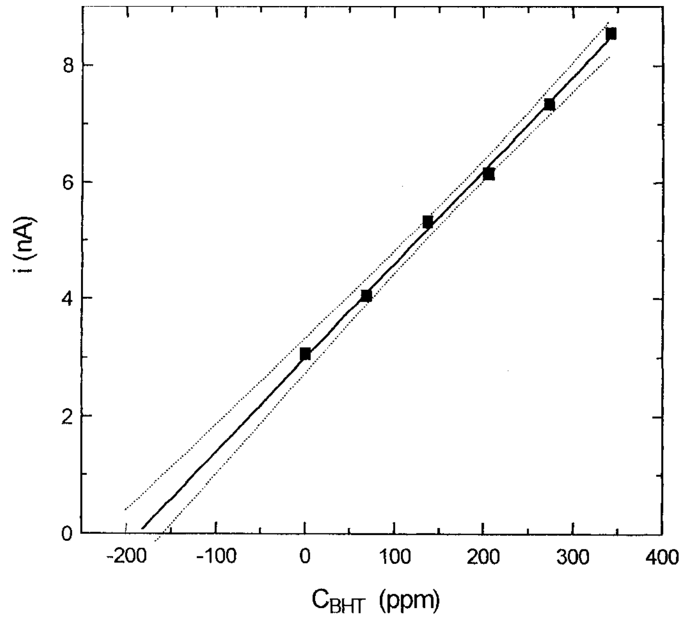

FIG. 3. Variations of peak current (i_p) from square-wave voltammetry (SWV) vs. ppm of BHT starting from an extract M2. Concentration of BHT in the initial oil sample: 205.00 ppm; recovery: 193.49 ppm (94.4%). Linear regression: $i_p = 3.03(0.08) + 0.016(0.001)\ C_{BHT}$; regression coefficient: 0.9989. See Figure 1 for other abbreviations.

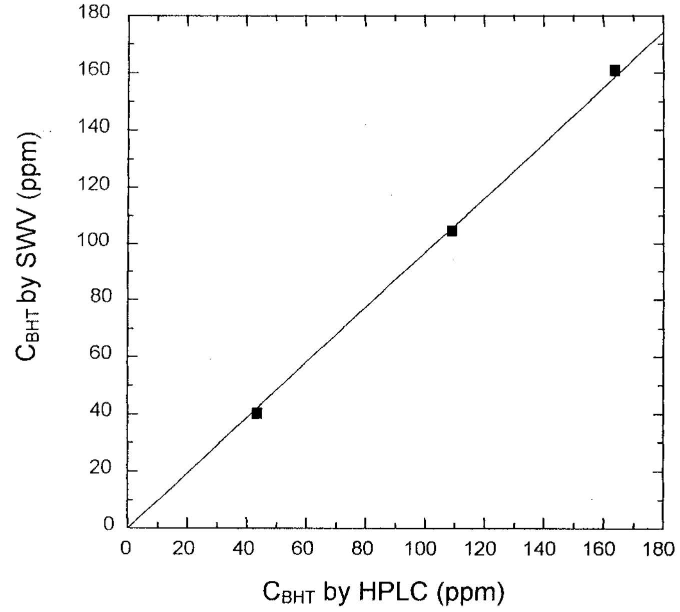

FIG. 5. Linear regression between BHT concentration values determined through SWV and high-performance liquid chromatography (HPLC) techniques on three given antioxidant–oil samples. Linear regression: C_{BHT} (from SWV) = 0.97(0.16) C_{BHT} (from HPLC) + 0.0(0.0). See Figures 1 and 3 for other abbreviations.

TABLE 2
The Recovery Percentage, Reproducibility Standard Deviation (S_R), and the Overall Reproducibility RSD$_R$ (%) of HPLC and SWV Results

	HPLC[a]	SWV[b]
Antioxidant	*R* (%) [S_R; RSD$_R$ (%)]	%R [S_R; RSD$_R$ (%)]
BHT	84.0 (4.54; 2.67)	91.33 (5.27; 2.82)
BHA	99.4 (6.61; 3.36)	93.68 (7.17; 3.69)

[a] Reference 27.
[b] This work. HPLC, high-performance liquid chromatography; SWV, square wave voltammetry. See Table 1 for other abbreviations.

rent methodology (27). The present work tends to show that SWV on carbon disk UME allows sensitive, reproducible, and faster determination of BHA, BHT, or antioxidant mixtures in oil–solvent solutions or after a simple extractive procedure with the advantage of a higher recovery percentage. This validates the methodology as an analytical alternative for antioxidant quantification.

ACKNOWLEDGMENTS

We would like to thank the Departamento de Química y Física-Facultad de Ciencias Exactas, Fisico-Químicas y Naturales and Facultad de Ingeniería for allowing this work to be performed. Financial support from SECYT (UNRC), CONICOR, and CONICET is gratefully acknowledged. We are indebted to Lilia Fernández for language assistance.

REFERENCES

1. Yusem, G., and P.N. Pintauro, The Electrocatalytic Hydrogenation of Soybean Oil, *J. Am. Oil Chem. Soc. 69*:399–404 (1992).
2. An, W., J.K. Hong, P.N. Pintauro, K. Warner, and W. Neff, The Electrochemical Hydrogenation of Edible Oils in a Solid Polymer Electrolyte Reactor. I. Reactor Design and Operation, *Ibid. 75*:917–925 (1998).
3. Bazinet, L., F. Lamarche, R. Labrecque, R. Toupin, M. Boulet, and D. Ippersiel, Electroacidification of Soybean Proteins for Production of Isolate, *Food Technol. 51*:52–60 (1997).
4. Transfeld, P., Electrofiltration Cuts Bleaching Earth Use, *INFORM 9*:502–506 (1998).
5. Kim, O.S., K. Maekawa, and K. Kusuda, Cyclic Voltammetry of Lipophilic Compounds in Oil. Direct Determination of Lipid Peroxide with a Carbon Paste Electrode, *J. Am. Oil Chem. Soc. 72*:299–303 (1995).
6. McBride, H.D., and D.H. Evans, Rapid Voltammetric Method for the Estimation of Tocopherols and Antioxidants in Oils and Fats, *Anal. Chem. 45*:446–449 (1973).
7. Clough, A.E., The Determination of Tocopherols in Vegetable Oils by Square-Wave Voltammetry, *J. Am. Oil Chem. Soc. 69*:456–460 (1992).
8. Yañez-Sedeño, P., J.M. Pingarrón, and L.M. Polo Diez, Determination of *tert*-Butylhydroxyanisole and *tert*-Butylhydroxytoluene by Flow Injection with Amperometric Detection, *Anal. Chim. Acta. 252*:153–159 (1991).
9. García, C.D., and P.I. Ortiz, Determination of *tert*-Butylhydroxytoluene by Flow Injection Analysis at Polymer Modified Glassy Carbon Electrodes, *Electroanalysis 10*:832–835 (1998).
10. Ruiz, M.A., M.G. Blázquez, and J.M. Pingarrón, Electrocatalytic and Flow Injection Determination of the Antioxidant *tert*-Butylhydroxyanisole at Nickel Phthalocyanine Polymer Modified Electrode, *Anal. Chim. Acta 305*:49–56 (1995).
11. Robards, K., and S. Dilli, Analytical Chemistry of Synthetic Food Antioxidants. A Review, *Analyst. 112*:933–943 (1987).
12. Huang, M.T., and T. Ferraro, Phenolic Compounds in Food and Cancer Prevention, in *Phenolic Compounds in Food and Their Effects on Health II—Antioxidant and Cancer Prevention*, edited by M.T. Huang, C.T. Ho, and C.Y. Lee, ACS Symposium Series 507, American Chemical Society, Washington, DC, 1992, pp. 8–34.
13. Valenzuela, B.A., and S. Nieto, Synthetic and Natural Antioxidants: Food Quality Protectors, *Grasas Aceites 3*:186–196 (1996).
14. *Official Methods of Analysis of AOAC International*, edited by P. Cunniff, 16th edn., 3rd rev., 1997, Vol. II, Gaithersburg, IUPAC-AOAC Method 42.2.02.
15. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, AOCS Press, Champaign, 1994, Method Ce 6-86.
16. Williams, D.E., Microelectrodes in Analysis, in *Microelectrodes: Theory and Applications*, edited by M.I. Montenegro, M.A. Queirós, and J.L. Daschbach, NATO ASI Series, Academic Publisher, Dordrecht, 1990, Vol. 197, pp. 415–428.
17. Cheek, G.T., and R. Mowery, Determination of Antioxidants in Lubricating Oils Using Ultramicroelectrodes, *Anal. Chem. 61*:1467–1469 (1989).
18. Aguí, M.L., A.J. Reviejo, P. Yañez-Sedeño, and J.M. Pingarrón, Analytical Applications of Cylindrical Carbon Fiber Microelectrodes. Simultaneous Voltammetric Determination of Phenolic Antioxidants in Food, *Ibid. 67*:2195–2200 (1995).
19. Ceballos, C., and H. Fernández, The Electroanalytical Determination of Antioxidants in Corn Oil Using Ultramicroelectrodes, *J. Braz. Chem. Soc. 6*:1–5 (1995).
20. Ceballos, C., and H. Fernández, Synthetic Antioxidants Determination in Lard and Vegetable Oils by the Use of Voltammetric Methods on Disk Ultramicroelectrodes, *Food Res. Int. 33/5*:357–365 (2000).
21. Fernández, H., and M.A. Zón, Heterogeneous Kinetics and Electroanalytical Determinations by Using Conventional Size Electrodes as Well as Ultramicroelectrodes, *Curr. Topics Electrochemi. 6*:111–137 (1998).
22. Osteryoung, J.G., and R.A. Osteryoung, Square Wave Voltammetry, *Anal. Chem. 1*:101A–110A (1985).
23. Whelan, D.P., J.J. O'Dea, J.G. Osteryoung, and K. Aoki, Square Wave Voltammetry at Small Disk Electrodes: Theory and Experiment, *J. Electroanal. Chem. 202*:23–36 (1986).
24. Ceballos, C., and H. Fernández, Electrochemical Quantification of Food Antioxidants by Pulse Techniques on Carbon Fiber Ultramicroelectrodes, *Proceedings of the XII Congreso de la Sociedad Iberoamericana de Electroquímica*, Mérida, Venezuela (1996).
25. Locatelli, C., and G. Torsi, Simultaneous Determination of Metals at Trace Level in a Multicomponent System. Application to Real Samples, *Electrochim. Acta 41*:2011–2017 (1996).
26. Page, B.D., High-Performance Liquid Chromatographic Determination of Seven Antioxidants in Oil and Lard: Collaborative Study, *J. Assoc. Off. Anal. Chem. 3*:727–745 (1983).
27. Page, B.D., Liquid Chromatographic Method for the Determination of Nine Phenolic Antioxidants in Butter Oil: Collaborative Study, *Ibid. 4*:765–779 (1993).
28. Código Alimentario Argentino, Ley No. 18284, Decreto No. 2126/71, edited by Marzocchi, Buenos Aires, Argentina, 1993.

[Received August 27, 1999; accepted April 9, 2000]