Spectrophotometric and Adsorptive Stripping Square Wave Voltammetric Determination of Iron in Olive Oils, as Complex with 5,5-Dimethylcyclohexane-1,2,3-trione 1,2-Dioxime 3-Thiosemicarbazone (DCDT)

T. Galeano Díaz, A. Guiberteau,* M. D. López Soto, and J. M. Ortiz

Department of Analytical Chemistry, University of Extremadura,
Avenida Elvas s/n, 06071 Badajoz, Spain

Two methods for the determination of iron in olive oil by spectrophotometry and by adsorptive stripping square wave voltammetry (Ad-SSWV) have been developed. These two methods are based on the formation of a 5,5-dimethylcyclohexane-1,2,3-trione 1,2-dioxime 3-thiosemicarbazone (DCDT)−iron(II) complex in strongly acid media. In both, iron is extracted from the olive oil by using HCl. Spectrophotometric determination of iron with DCDT is based on the feature that the DCDT−Fe complex shows an absorbance maximum at 550 nm. A calibration graph has been constructed from 0 to 4000 ng mL^{-1}, and the detection limit was 115 ng mL^{-1} (57 ng g^{-1} in olive oil). On the other hand, the voltammetric determination of the metal is based on the appearance of a peak due to an adsorptive reductive process of the complex that it is observed when the Ad-SSWV technique is used. A calibration graph has been constructed from 0 to 30 ng mL^{-1}, and the detection limit was 0.55 ng mL^{-1} (13.75 ng g^{-1} in olive oil according to the proposed procedure).

KEYWORDS: Iron; olive oil; spectrophotometry; stripping voltammetry

INTRODUCTION

It is widely known that some of the most important quality parameters of the olive oil are the peroxide values, K_{232} and K_{270}. These inform us about the oxidation state of the olive oil.

The causes that initialize the oxidation process may be diverse. Among them, we can remark that transition metals, such as iron and copper, are known to be prooxidant factors because they catalyze the decomposition of hydroperoxides. This decomposition is accompanied by the formation of new radicals, and then reaction proceeds easily (*1*, *2*).

There are few metals reported to be present in olive oil: copper, iron, nickel, manganese, cobalt, chromium, tin, and lead, but the most abundant ones are iron (between 0.5 and 3 ppm) and copper (between 0.001 and 0.2 ppm) (*2*).

Most of the procedures that we have found in the literature for the determination of iron in olive oil are based on the use of AAS techniques involving an ashing pretreatment of the sample before the analysis (*3*) or just a dilution of the olive oil sample in methyl isobutyl ketone (*4*). Other techniques such as ICP AES (*5*) are also used and, more rarely, spectrophotometric (*6*) and voltammetric (*7*) techniques.

In this paper we propose two methods to determine iron in olive oil. Both of them involve the extraction of the metal from olive oil samples by using hydrochloric acid (*6*), and then 5,5-

dimethylcyclohexane-1,2,3-trione 1,2-dioxime 3-thiosemicarbazone (DCDT) reagent is added to the solution obtained. Once the complex DCDT−Fe is formed, the analysis is carried out. DCDT reacts with Fe(II) and Fe(III), in strongly acid media, giving rise to a violet complex of Fe(II) (*8*). It is important to emphasize that DCDT has a great selectivity, and the only metal that could interfere is Co(II) (*8*). In a previous paper, DCDT reagent has been used to analyze iron in wines, vegetables, and minerals (*8*) and in acids (*12*), by spectrophotometry. It has been also used to determine Fe in acids, waters, fruit juices, and wines by polarography (*9*).

EXPERIMENTAL PROCEDURES

Apparatus. A Unicam model UV 500 spectrophotometer with a 1.0 cm path length quartz cell and double beam scanning was used.

The Crison basic 20 pH-meter had a combined SCE-glass electrode.

An Autolab AUT 12.v Ecochemie Polarecord was used in combination with a Metrohm VA-663 polarographic stand, with a three-electrode system with hanging mercury drop electrode as a working electrode, a Ag/AgCl-saturated KCl reference electrode, and a Pt wire auxiliary electrode. The system is monitored by the General Purpose Electrochemical System (GPES4) version 4.0 (Ecochemie, Ultrecht, The Netherlands) software package. Golden Software (Grapher, v. 1.32) (Golden, CO) was used for the transformation of initial signals.

Reagents. DCDT was synthesized according to the method of Haas (*10*), modified as described later (*8*).

Stock standard Fe(III) solution (1000 mg L^{-1}) was from Panreac (Barcelona, Spain). A standard solution of Fe(II) (1000 mg L^{-1}) was

prepared from ammonium iron(II) sulfate hexahydrate (Merck, Darmstadt, Germany). Organometallic IPC-XRF standard iron (4998 μg/g) was from AccuStandard, Inc. (New Haven, CT). All subsequent iron solutions were prepared by dilution.

All other chemicals were of analytical reagent grade or better.

Recommended Procedure for the Determination of Iron in Olive Oil by Spectrophotometry. A suitable amount of olive oil, containing between 5 and 100 micrograms of iron (~50 g), and 10 mL of 5 M HCl are introduced into a 100 mL extraction flask. Then the mixture is shaken for 1 min. The acidic layer is placed into a 25 mL volumetric flask. The organic layer is extracted again under the same conditions described above. Later 1 mL of 1% DCDT in dimethylformamide is added to the extracts, and the solution is diluted to the mark with deionized water. After filtering through a 0.45 micra nylon membrane filter, the absorbance was measured at 550 nm by using a 1.0 cm cell. The blank is prepared from an olive oil sample (known to contain a low amount of iron), by repeated extractions with 2 M HNO$_3$ (*11*) and in that way we obtain an olive oil sample free of metal. The blank is treated following the procedure described above, and olive oil samples are measured against the blank. The standard addition method is used: olive oil samples are fortified with iron, at different levels of concentrations, using a solution of an organometallic standard iron, dissolved in a metal-free olive oil.

Recommended Procedure for the Determination of Iron in Olive Oil by Adsorptive Stripping Square Wave Voltammetry (Ad-SSWV). A suitable amount of olive oil containing between 50 and 1500 ng of iron (~1−2 g) is extracted with 5 M HCl (10 mL × 2), shaking the mixture for 1 min each time, as was described for the spectrophotometric method. The extracts are placed into a 50 mL volumetric flask. HCl is added to obtain a final concentration of 2.5 M HCl, and 1.2 mL of 0.04% DCDT in ethanol is added. The solution is diluted to the mark with deionized water. The sample is stirred while accumulation takes place. After the accumulation period, the stirring is stopped and, after 15 s of equilibrating time, the voltammogram of the sample is recorded. The instrumental conditions are as follows: tac = 35 s, Eac = −0.02 V, Ei = −0.100 V, frequency = 165 Hz, step potential = 5 mV, amplitude = 50 mV, stirring rate = 500 rpm. From the analytical signal, peak current, or Ip, the concentration is calculated by using a suitable prepared calibration graph.

RESULTS AND DISCUSSION

Spectrophotometric Determination of Iron in Olive Oil. HCl has been chosen as the most suitable acid, because the formation of chloro complexes of Fe favors the extraction of this metal from the samples. Although HNO$_3$ is a very good extractant for Fe, with this acid the color of the Fe−DCDT complex does not develop, probably due to the oxidizing properties of this acid. To obtain the optimum concentration of acid to extract iron from olive oil samples, we varied the HCl concentration from 1 to 8 M. In all cases, 10 mL of HCl was added to 50 g of olive oil samples into an extraction flask. The extract was collected into a 25 mL volumetric flask. To avoid the influence of HCl concentration over the formation of the complex, the final acid concentration in the volumetric flask was 3.2 M (*12*). **Figure 1** shows that absorbance at 550 nm is maximum when 5 M HCl is used. It was also observed that metal recovery is maximum when two extraction steps are made. Therefore, two extraction steps with 5 M HCl were selected for the following experiments.

Shaking time was optimized by varying it from 30 s to 3 min. It was observed that the absorbance value of the extracted solution, at 550 nm, remains constant when the mixture was shaken for 1, 2, or 3 min. For the following experiments, samples were shaken for 1 min.

With regard to the influence of DCDT concentration, the absorbance remains constant with concentrations of reagent above 20-fold molar excess; therefore, 1 mL of 1% reagent solution in dimethylformamide in a final volume of 25 mL was
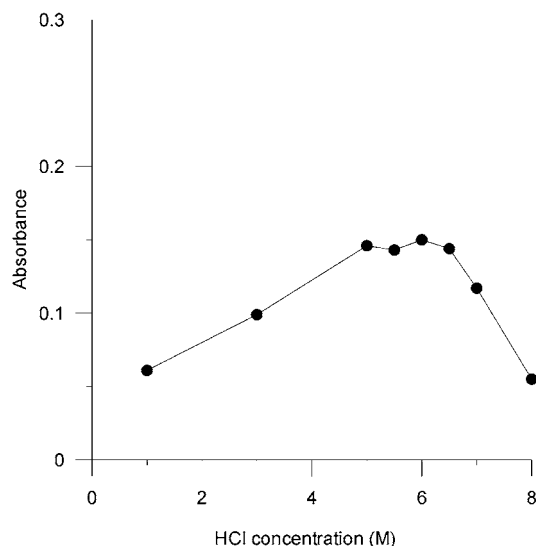

Figure 1. Influence of acid concentration used in the extraction of iron from olive oil.
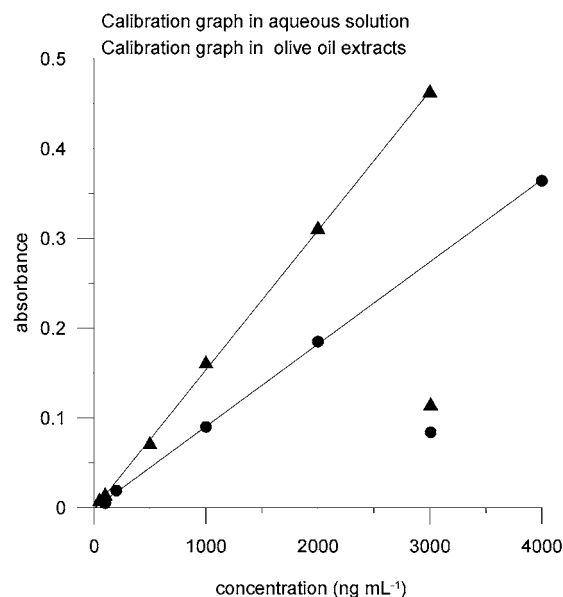

Figure 2. Influence of iron concentration on the absorbance values when different iron standards are used.

selected for the analytical procedure. The order of reagent addition was immaterial.

Calibration and Precision. To study the possibility of matrix interferences produced by olive oil, we first constructed a calibration graph by using aqueous standard solutions of Fe(III). Later, we constructed another calibration graph, in which samples of a metal-free olive oil were fortified with organometallic standard Fe, at different levels of concentrations. Those calibration graphs were compared, and significant differences between the slopes were found (**Figure 2**). This indicated to us that it is necessary to apply a standard addition method to determine iron by spectrophotometry.

Fe(III) is reduced by the reagent giving rise to a Fe(II) complex. As iron in olive oil is present as Fe(III), it was proven that there were not variations in the formation of the complex when a solution of Fe(III) was used instead of a Fe(II) solution.

A calibration plot was constructed by adding different amounts of organometallic standard Fe to 50 g of a metal-free olive oil. The iron concentration in olive oil samples was varied
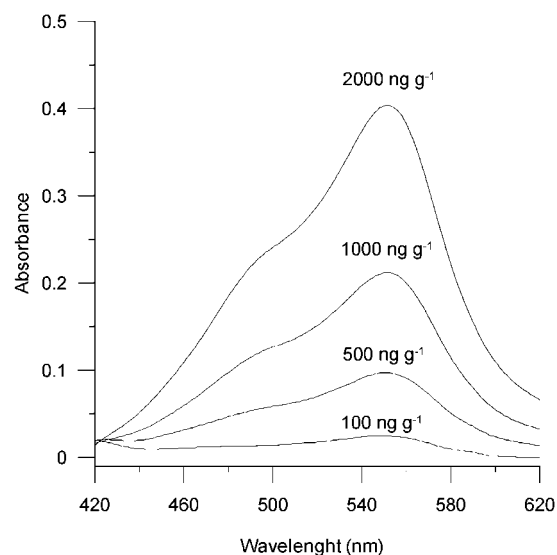
Determination of Iron in Olive Oil

J. Agric. Food Chem., Vol. 51, No. 13, 2003 **3745**

Figure 3. Spectra of Fe–DCDT obtained for Fe concentrations in olive oil between 100 and 2000 ng g^{-1}.

Table 1. Determination of Iron in Olive Oil

	iron found (ng g^{-1}) ± standard deviation		
olive oil	spectrophotometry	AAS	Ad-SSWV
1	296 ± 32	318 ± 21	324 ± 9
2	658 ± 29	692 ± 34	716 ± 14
3	424 ± 66	427 ± 27	454 ± 1
4	254 ± 19	333 ± 33	308 ± 7
5	445 ± 34	527 ± 35	483 ± 5

from 100 to 2000 ng g^{-1}, and samples were prepared in triplicate. The proposed method was followed to obtain the extracts. The absorbance at the maximum was measured and plotted against iron concentration in olive oil (**Figure 3**). The calibration curve was $A = 9.16 \times 10^{-5}C - 9.06 \times 10^{-4}$ (C being iron concentration, in ng mL^{-1}, in the final solution). The correlation coefficient was 0.9978, and the detection limit (*13*) was 115 ng mL^{-1} (57 ng g^{-1} in olive oil).

The relative error of the method was estimated from nine samples of olive oil containing 500 ng g^{-1} of iron and treated as described in the procedure; measurements were made using optimal conditions. The mean value of absorbance was 0.088 au, and the relative standard deviation was 8.38%.

Applications. The proposed method has been applied to the determination of Fe in several samples of olive oil. These results have been compared to those obtained by atomic absorption spectrophotometry (*14*) (**Table 1**). This AAS method consisted of dissolving olive oil in methyl isobutyl ketone in a ratio of 1:4 (v/v). The analysis takes place in an AAS apparatus with a graphite furnace with a programmed temperature ramp in six steps. We modified this method for our particular case and instead of 1:4 (v/v), we used 1:20 (w/v) olive oil/methyl isobutyl ketone.

Ad-SSWV Determination of Iron in Olive Oil. A method for the determination of iron in acids, wines, and fruits juices by differential pulse polarography, based on formation of a DCDT complex, was found in the literature (*9*). This method was used for the determination of iron in the range of 0.02−4.5 mg L^{-1}, in the presence of 2.5 M HCl, 0.008% DCDT solution, and 20% ethanol. The polarogram of the sample was recorded from 0.0 to −0.5 V, after 10 min of deoxygenation with N$_2$. The electrochemical reduction of the Fe(II)−DCDT complex is irreversible (*9*) in these conditions.
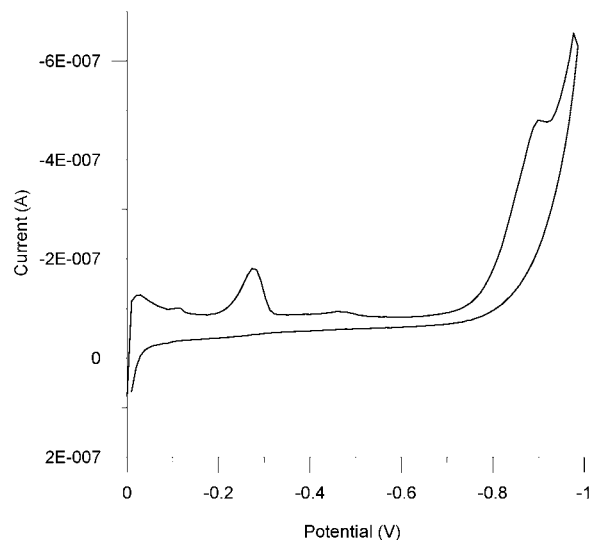

Figure 4. Cyclic voltammogram of Fe–DCDT (300 ng mL^{-1} of Fe, 1.6 × 10^{-3}% DCDT, 2.5 M HCl) 4% (v/v) ethanol with a scan rate of 200 mV/s.

In this paper, we have examined the reduction peak of the Fe(II)−DCDT complex by cyclic voltammetry to check its possible adsorptive processes. We have recorded the voltammograms of a solution containing 300 ng mL^{-1} of Fe, 1.6 × 10^{-3}% DCDT solution in ethanol (4% v/v), and 2.5 M HCl, with different scan rates, between 20 and 200 mV s^{-1}. The symmetric shape of the cathodic peak, as well as the linear variation of Ip with the scan rate, proved that this is effectively a peak due to an adsorptive−reductive process. It was also observed that no signal appears in the reverse scan, indicating the nonreversible character of the process (**Figure 4**).

We have studied the influence of chemical and instrumental variables over Ip in Ad-SSWV for the complex Fe−DCDT (100 ng mL^{-1} of Fe). First, dimethylformamide and ethanol were tested as solvents for DCDT. For equal DCDT concentrations, the peak current of the complex is greater when DCDT is dissolved in ethanol than when it is dissolved in DMF. Afterward, the DCDT concentration was varied from 3.2 × 10^{-4} to 24 × 10^{-4}%, and it was found that the peak current remained constant with concentrations of reagent above 20-fold molar excess. A 9.6 × 10^{-4}% concentration of DCDT (0.6 mL of 0.04% solution in ethanol) was selected for subsequent experiments.

The acid concentration was varied from 1 to 5 M. The peak potential (Ep) of the complex was displaced toward less negative potentials, as the HCl concentration increased. Peak current remained constant for HCl concentrations between 2 and 4 M (**Figure 5**). The HCl concentration of 2.5 M was selected as the most suitable for the determination of iron by Ad-SSWV, taking into account Ip and Ep.

With regard to the instrumental variables, the accumulation time (tac) was studied for solutions of 10, 50, and 100 ng mL^{-1} of iron, and the tac was varied from 30 to 60 s (**Figure 6**). From the ANOVA of the results, it was found that there is linearity up to 40 s at least for the iron concentrations we have examined. For the following experiments, we used 35 s as tac because this tac value ensured that the electrode is not going to be saturated.

The accumulation potential (Eac) was varied from −0.100 to 0.02 V. When the Eac was equal to the initial potential (Ei), utilized to record the voltammogram, there was no zone in which the Ip remained constant with the variation of the Eac, but when the Ei was −0.100 V and the Eac was varied, it was found that
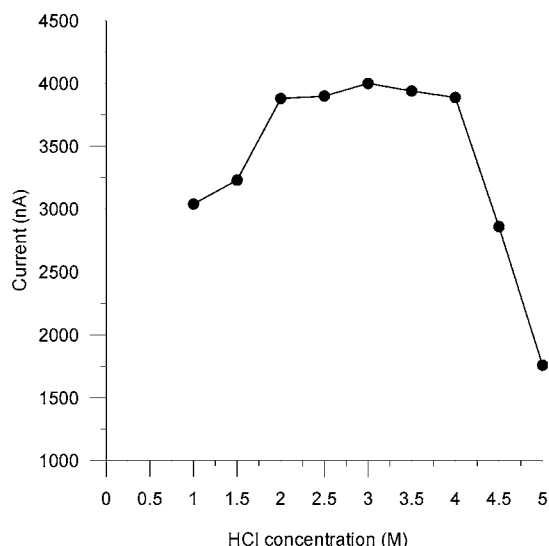
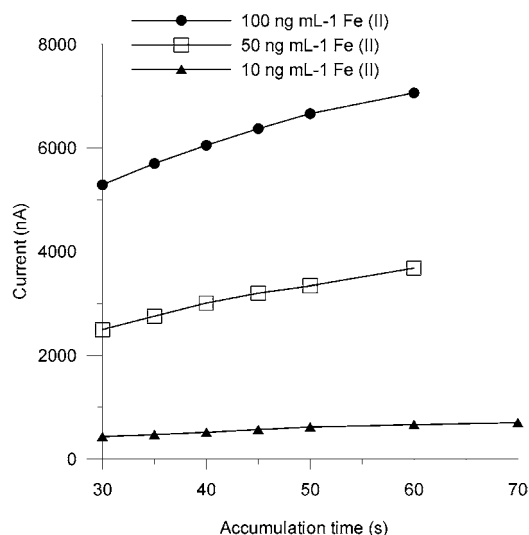**Figure 5.** Influence of acid concentration on the Fe–DCDT peak current.

Figure 6. Influence of accumulation time on the Fe–DCDT peak current for different concentrations of Fe.

the Ip remained constant for Eac values from -0.01 to -0.03 V. When Ei was changed to -0.150 V, there was also a zone of Eac for which the Ip was constant, but in this case, a decrease of the Ip occurs, in relation to the other case. Therefore, Eac $= -0.02$ V and Ei $= -0.100$ V were considered to be the most appropriate values for the analysis.

The peak current of the complex increased linearly with an increase in the frequency from 25 to 200 Hz. In this work we selected 165 Hz for the analysis. As before, the peak current of the complex also increased linearly with an increase in the step potential from 2 to 10 mV, but, as the step potential increased, the peak width also increased. Thus, 5 mV was selected as the most suitable step potential value, because it offered the higher Ip and the best peak definition. When the amplitude was varied from 20 to 100 mV, a maximum in the Ip was observed, which corresponded to an amplitude value of 50 mV. A 500 rpm was selected as stirring rate.

Calibration and Precision. Once all of the parameters that could affect the determination of Fe were optimized, the variation of peak current with the concentration of Fe was studied. The Fe concentration was varied from 2 to 30 ng mL^{-1}. There were no differences in Ip and Ep or in the behavior of
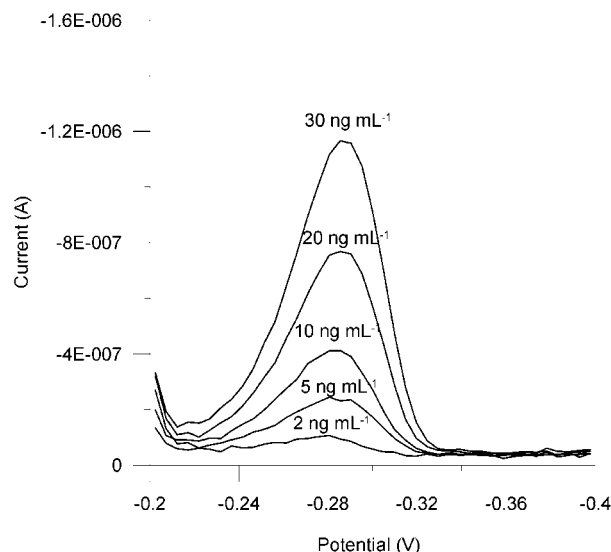

Figure 7. Ad-SSW voltammograms of Fe–DCDT obtained for Fe concentrations between 2 and 30 ng mL^{-1}.

the complex between either a solution of Fe(II) or a solution of Fe(III); however, for the Fe(II) solution $\sim$10 min was necessary for the total formation of the complex, but for the Fe(III) solution $\sim$50 min was necessary for the complete formation of the complex.

First, calibration plots were constructed using aqueous standard solutions, and, later, a new calibration plot was constructed with olive oil samples fortified with organometallic standard iron. Voltammograms of the calibration plot are shown in **Figure 7**. The slopes of both plots were compared, and no significant differences were observed.

For the calibration plot in aqueous solution: Ip $= 35.66C + 0.22$ (C being the iron concentration in ng mL^{-1}). Correlation coefficient $= 0.9996$, and detection limit (*13*) $= 0.37$ ng mL^{-1}.

For the calibration plot in olive oil extracts: Ip $= 35.54C + 13.55$ (C being the iron concentration in ng mL^{-1}). Correlation coefficient $= 0.9982$, and detection limit (*13*) $= 0.55$ ng mL^{-1} (13.75 ng g^{-1} in olive oil according to the proposed procedure).

This indicated to us that in this case there was no matrix effect; thus, the determination of iron may be direct. The relative error of the method was estimated with seven samples of olive oil containing 250 ng mg^{-1} of iron. Samples were analyzed, according to the method described in the procedure, and measurements were made, using optimal conditions. The mean value of peak current was 329 nA, and the relative standard deviation was 9.15%.

Interference from Other Ions. As mentioned above, Cu(II) is the only metal that could be found, in relative abundance, in olive oil, together with iron. The influence of Cu(II) was investigated by preparing various solutions containing 40 ng mL^{-1} of Fe and different concentrations of Cu(II), in a range from 0 to 500 ng mL^{-1}. Once the solutions were measured under the optimal conditions, it was observed that Cu(II) caused interference in a ratio of 1:2.5, Fe/Cu(II), which is not normally present in olive oil.

Application. The proposed method was applied to the determination of Fe in several samples of olive oil. These results were compared with those obtained by AAS (**Table 1**).

In conclusion, we can point out that the results obtained for the determination of Fe in olive oil, by spectrophotometry and by Ad-SSWV, are very good compared to those obtained by AAS. The detection limit for the Ad-SSWV method is much

Determination of Iron in Olive Oil

J. Agric. Food Chem., Vol. 51, No. 13, 2003   **3747**

lower than that for the spectrophotometric method, but the RSD values for the two methods are essentially the same.

These methods are simpler and easier to use than other methods, voltammetric (*7*) and spectrophotometric (*6*), found in the literature, because in these other methods sample preparation is tedious. On the other hand, some of the AAS methods are very easy to carry out because only dilution of the sample is necessary (*4*, *9*). However, a graphite furnace must be employed, and the cost of this technique is greater than those of the spectrophotometric and voltammetric techniques.

ACKNOWLEDGMENT

LITERATURE CITED

(1) De Leonardis, A.; Macciola, V.; Palmucci, M. Iron Content in Virgin Olive Oil Produced by Classical System. *Ind. Aliment.* **2000**, *39* (396), 1119−1122.

(2) Boskou, D. *Olive Oil, Chemistry and Technology*; AOCS Press: Champaign, IL, 1996; 115 pp.

(3) Garrido, M. D.; Diaz, I.; Hardisson, A. Concentration of Metals in Vegetable Edible Oils. *Food Chem.* **1994**, *50* (3), 237−243.

(4) Martin-Polvillo, M.; Albi, T.; Guinda, A. Determination of Trace Elements in Edible Vegetable Oils by Atomic Absorption Spectrophotometry. *J. Am. Oil Chem. Soc.* **1994**, *71* (4), 347−353.

(5) Murillo, M.; Benzo, Z.; Marcano, E.; Gomez, C.; Garaboto, A.; Marin, C.; Determination of Cooper, Iron and Nickel in Edible Oils using Emulsified Solutions by ICP AES. *J. Anal. At. Spectrom.* **1999**, *14* (5), 815−820.

(6) Coupkova, M.; Winterova, L.; Mach, I. Modified Extraction Method of Sample Preparation to Determine Iron Content in Refined Oils and Non-emulsified Hardened Fats. *Sb UVTIZ, Potravin. Vedy* **1988**, *6* (1), 29−33.

(7) Wu, B. F.; Ye, H. Y.; Zhao, D. H. Stripping Voltammetric Determination of Zinc and Iron in Cereal and Oil Products. *Lihua Jianyan, Huaxue Fence* **2000**, *36* (9), 398−399.

(8) Salinas, F.; Jiménez, J. C.; Galeano, T. Spectrophotometric Determination of Iron in Wines, Foods, and Minerals with 5,5-Dimethylcyclohexane-1,2,3-trione 1,2-Dioxime 3-thiosemicarbazone. *Anal. Chem.* **1986**, *58*, 824−827.

(9) Vázquez, M. E.; Jiménez, J. C.; Callejón, M.; Guiraúm Pérez, A. Differential Pulse Polarographic Determination of Iron in Acids, Waters, Fruit Juices and Wines. *Analyst* **1994**, *119*, 1571−1574.

(10) Haas, P. iso Nitroso- and Nitro-dimethyldihydroresorcin. *J. Chem. Soc.* **1907**, *91*, 1433−1448.

(11) Calapaj, R.; Chiricosta, S.; Saija, G.; Bruno, E. Method for the Determination of Heavy Metals in Vegetable Oils by Graphite Furnace Atomic Absorption Spectrophotometry. *At. Spectrosc.* **1988**, *9*, 107−109.

(12) Lemus Gallego, J. M.; Galeano Díaz, T.; Jiménez Sánchez, J. C.; Spectrophotometric Determination of Iron in Acids. *Analyst* **1988**, *113*, 1341−1342.

(13) Long, G. L.; Winefordner, J. D. *Anal. Chem.* **1983**, *55*, 713A.

(14) Minoia, C.; Caroli S. *Applications of Zeeman Graphite Furnace Atomic Absorption Spectrometry in the Chemical Laboratory and Toxicology*; Pergamon Press: New York, 1992; 257 pp.