Talanta 99 (2012) 104–112

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

# Application of the extraction induced by emulsion breaking for the determination of chromium and manganese in edible oils by electrothermal atomic absorption spectrometry

Nicolle F. Robaina, Daniel M. Brum, Ricardo J. Cassella *

*Departamento de Química Analítica, Universidade Federal Fluminense, Outeiro de S.J. Batista s/n, Centro, Niterói/RJ, 24020-141, Brazil*

ARTICLE INFO

*Article history:*
Received 28 February 2012
Received in revised form
14 May 2012
Accepted 16 May 2012
Available online 24 May 2012

*Keywords:*
Edible oils
Extraction induced by emulsion breaking
Metals
ETAAS

ABSTRACT

This work reports the optimization of a method, based on the extraction induced by emulsion breaking, for the determination of trace concentrations of Cr and Mn in edible oils by electrothermal atomic absorption spectrometry (ETAAS). In the method, a water-in-oil emulsion was prepared by mixing the oil sample with an acid solution ($HNO_3$) of Triton X-114 to allow the intense contact between the sample and the extractant acid solution. Afterwards, the emulsion was broken by heating and the acid aqueous phase deposited in the bottom of the flask was collected for the determination of the metals of interest. The method was optimized by studying the influence of several parameters such as the concentration of $HNO_3$ and the emulsifier agent (Triton X-100 and Triton X-114) in the extractant solution. The best results were verified when the procedure was performed with 5 mL of the sample and 1 mL of the extractant solution containing 15% m/v of Triton X-114 and 2.8 mol $L^{-1}$ of $HNO_3$. Also, the fastest emulsion breaking was verified when the emulsions were heated at 90 °C. In these conditions, the emulsions were broken in approximately 10 min. The quantification of Cr and Mn in the extracts was carried out by external calibration with aqueous standard solutions, which simplified the procedure. The limits of detection for the determination of Cr and Mn in the oil samples were 66 and 36 ng $L^{-1}$, respectively, and the limits of quantification were 219 and 120 ng $L^{-1}$, respectively. The developed method was applied in the determination of Cr and Mn in twelve samples of edible oils produced with different oleaginous. Recovery tests were performed to attest the accuracy of the method, being observed recovery percentages in the range of 86–115%.



## 1. Introduction

Nowadays, the development of suitable tools for the verification of the quality and safety of foodstuff is an important issue in the food industry. In this universe, the quality control of edible oils can be highlighted, since they play a fundamental role in the human nutrition and are largely consumed worldwide [1].

Classically, the verification of the quality of the edible oils is related to the determination of some chemical parameters such as acidity, concentration of peroxides and fatty acids composition. Nevertheless, the determination of major and trace elements is also necessary, especially because of the important metabolic role played by some metals in the human organism and also due to toxicity issues. Another aspect to be considered is the influence of some metallic elements on the stability of the oils, since they act as catalysts in the oxidative reactions of the oils that generate peroxides, cetones, aldehydes and other substances with pathogenic effects [2].

Different approaches have been explored to conduct the determination of metals in edible oils by atomic spectrometry. When possible, the direct injection of the samples (or samples diluted with organic solvents) into the instruments is a good option, since minimum sample handling is required, which avoids problems of contamination and losses [3]. However, in several cases, the direct analysis is not possible because the complex organic matrix affects the performance of the spectrometers and makes the calibration procedures very laborious. Additionally, sometimes, the methods require the handling of toxic solvents for the dilution of the samples in order to decrease its viscosity [4–7].

Alternative strategies have been developed to overcome the problems observed in the direct analysis of edible oils and other types of oil samples. The most common alternative is the total digestion of the samples using dry [8,9] or wet ashing [10–15] procedures. In these methods, the organic matrix is mineralized and the analytes are transferred to an aqueous solution, where they are easily measured by atomic spectrometric techniques.

* Corresponding author. Tel.: +55 21 2629 2222; fax: +55 21 2629 2143.
*E-mail address:* cassella@vm.uff.br (R.J. Cassella).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.05.025

In general, these procedures are time-consuming, laborious and prone to errors due to the contamination of the samples and losses of the analytes [16].

Some other procedures, based on the modification of the organic matrix, through the formation of emulsions or micro-emulsions, are reported [17–21]. These procedures present the same problem observed in the direct analysis of the oils, since the organic matrix is not destructed before introduction into the spectrometer. However, they facilitate some points of the analytical process, especially those related to the calibration procedure and the lack of stability of the standard solutions prepared with organometallic compounds.

Extraction methods with diluted solutions of acids seem to be suitable for the pretreatment of the samples of edible oils for the elemental analysis. They employ less aggressive reagents, demand lower time than total digestion and allow the use of aqueous standards for calibration, which simplify the procedure. Despite of these advantages, only few papers were found in the literature regarding the extraction of metals from edible oils [22–25], maybe due to the difficult to achieve a convenient contact (and extraction) between the oils and the acid aqueous extractant solutions, which are practically immiscible. In this field, some papers can be cited. Leonardis et al. [22] proposed a method for the extraction of Cu and Fe from edible oils using a 10% v/v $HNO_3$ solution. The oil samples were heated at 50 °C to decrease the viscosity and allow a proper mixing with the aqueous extractant solution. The main disadvantage of the method was the high time required to achieve the extraction, which was 2 h. The metals were measured by electrothermal atomic absorption spectrometry (ETAAS) and the method was applied in the analysis of edible oils of different origins (olive, soybean and sunflower). Pehlivan et al. [23] employed, with success, exactly the same procedure for the determination of nine metals (Cu, Fe, Mn, Co, Cr, Pb, Cd, Ni, and Zn) in seventeen samples of vegetable oils obtained from different oleaginous.

Anwar et al. [24] also developed an extraction method using a 2 mol $L^{-1}$ $HNO_3$ solution under ultrasonic irradiation. The method required the dilution of 5 g of the samples with 10 mL of $CCl_4$, which made it very aggressive for the analyst and the environment. Ooms and Van Pee [25] proposed the extraction of metals from corn oil using an EDTA solution. After the extraction, the determination of the metals was carried out by ETAAS.

The goal of this work was to propose a new method for the extraction of metals (Cr and Mn) from edible oils employing the extraction induced by emulsion breaking, which was successfully applied in the extraction of various metals (Cu, Fe, Ni, Pb, Zn, Al, Mn, Sn and V) from diesel oil [26–28]. In these works, the determination of the metals in the extracts was performed by different analytical techniques (FAAS, ETAAS and ICP-MS), which proved the versatility of the methodology. Several advantages can be pointed out when the extraction induced by emulsion breaking is employed such as the possibility to perform the calibration with aqueous standard solutions, the low time required to complete the extraction process and the preconcentration of the analytes in the extractant aqueous phase.

## 2. Experimental

### 2.1. Apparatus

The determination of Cr and Mn in the solutions (extracts and standard solutions) was carried out with a Varian (Mulgrave, Australia) graphite furnace atomic absorption spectrometer, model AA240Z, equipped with a Varian GTA 120 atomizer unit and a Varian PSD 120 auto sampler. Graphite tubes with L'vov platform made of pyrolytic graphite (Varian part no. 63-100026-00) were used. Background correction was done with a Zemman-effect based corrector, which was operated with a constant magnetic field strength fixed at 0.8 T. Chromium was measured at 357.9 nm with a nominal spectral resolution of 0.2 nm and using an individual hollow cathode lamp operated at 7.0 mA. Manganese was measured at 279.5 nm with a 0.2 nm nominal spectral resolution and using a hollow cathode lamp operated at 5.0 mA.

Argon with 99.99% (Linde Gases, Macaé, Brazil) of purity was employed as protective gas for the graphite tube.

The emulsion breaking was induced by heating using a water bath with a temperature control ($\pm 0.1$ °C), model NT 247, supplied by Nova Técnica (São Paulo, Brazil).

The total digestion of the olive oil sample ($S_{13}$) used in the preparation of the reference sample was carried out with an Anton Paar Multiwave 3000 (Graz, Austria) microwave oven equipped with PFA vessels.

### 2.2. Reagents and solutions

The water used throughout the experimental work was purified in a Direct-Q 3 System (Millipore, Milford, MA, USA) and always had a resistivity higher than 18.2 MΩ cm.

Hexane (HPLC grade), supplied by Tedia (Fairfield, OH, Brazil), was used as diluent in the preparation of oil-based standard solutions.

Aqueous stock solutions of chromium and manganese with a concentration of 1,000 μg $mL^{-1}$ were furnished by SPEX (Metuchen, NJ, USA). The analytical solutions employed in the present work were prepared by suitable dilution of the stock solutions with purified water.

Oil-based stock solutions of chromium and manganese with a concentration of 1,000 μg $g^{-1}$ were furnished by Conostan (Houston, TX, USA). The oil-based standard solutions of the selected metals were prepared by suitable dilution of the stock solution with hexane.

The acid Triton X-114 (Acros Organics, St. Louis, USA) and Triton X-100 (Tedia, Fairfield, OH, USA) solutions employed for emulsification purposes were prepared by dissolving suitable masses (according to the experiment) of each surfactant in exactly 100 mL of $HNO_3$ solution. The desired concentration of the $HNO_3$ solution was established according to the experiment. The concentrated nitric acid used to prepare such solutions had trace metal grade and was supplied by Tedia (Fairfield, OH, USA).

The vegetable oil employed in the experiments of optimization, named reference sample, was prepared by mixing 5% v/v of a sample of olive oil ($S_{13}$), containing $112 \pm 17$ μg $L^{-1}$ of Mn, with 95% v/v of a sample of soybean oil, containing unknown concentrations of Cr and Mn. It was stored in a low-density polyethylene flask and kept in a light-free place at laboratory ambient temperature ($23 \pm 1$ °C). The reference sample yielded measurable signals after application of the extraction induced by emulsions breaking and was suitable for the optimization of the methodology. The content of Mn in the olive oil ($S_{13}$) was determined by ETAAS, in triplicate, after its total dissolution with concentrated $HNO_3$ in a closed vessel microwave oven.

All the samples analyzed by the developed method were purchased in supermarkets of the city of Niterói, Rio de Janeiro, Brazil. They were also stored in a light-free place at ambient temperature.

### 2.3. Extraction induced by emulsion breaking (EIEB) procedure

The extraction of Cr and Mn from edible oils was carried out by the extraction induced by emulsion breaking using the conditions

optimized in the present work. The first step of the extraction procedure was the formation of the water-in-oil emulsion by the vigorous mixing of 5 mL of oil with 1 mL of a solution containing Triton X-114 and $HNO_3$. The emulsion was prepared in a capped polyethylene tube of 13 mL. Afterwards, the tube containing the emulsion was transferred to the water bath maintained at $90 \pm 1$ °C, where it was heated until the emulsion was broken, which took approximately 10 min. The emulsion breaking yielded two phases: (A) an upper phase, containing the edible oil and (B) a lower phase, which was the aqueous solution containing the metals extracted by the acid (Fig. 1).

After the emulsion breaking, the flask was taken out of the water bath and the separation of the phases was carried out. Firstly, the upper phase (edible oil) was transferred to another flask with the aid of a micropipette. Residual amounts of the oil remained in the tube, but they were not collected together with the aqueous phase. The collection of the aqueous phase was also performed with a micropipette. In this case, exactly 0.5 mL of the aqueous phase was transferred to the vial of the ETAAS for the measurement of the metals under study or, when necessary, transferred to a volumetric flask for convenient dilution. It is important to notice that the dilution ratio varied according to the metal because of the different sensitivities of the ETAAS technique for each metal and their actual concentration in the samples.

### 2.4. Determination of the metals in the extracts by ETAAS

The measurement of both Cr and Mn in the extracts or in the standard solutions was performed by injecting 20 µL of the solutions into the graphite tube. The addition of chemical modifiers was not necessary because the temperatures employed in the pyrolysis step were not high enough to promote significant volatilization of the analytes. The measurements were always carried out in the integrated absorbance mode. The Table 1 presents the general temperature program.

### 2.5. Material decontamination

Glassware and plastic flasks were decontaminated in order to avoid any contamination of the acid extracts and other solutions.

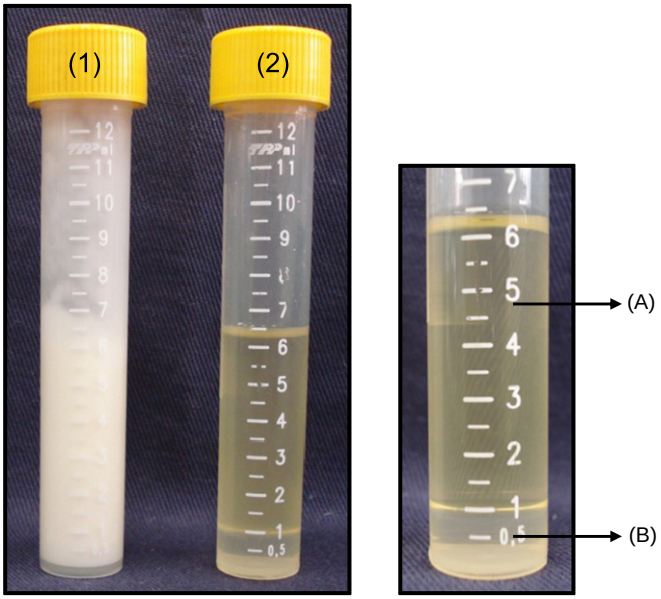


**Fig. 1.** Emulsion (1) before and (2) after breaking by heating at 90 °C. (A) upper phase containing the vegetable oil; (B) lower phase containing an acid aqueous with the metals that were extracted.

**Table 1**
Programs of temperature employed in the analysis of the aqueous extracts obtained by the proposed extraction method by ETAAS.

| Step | T(°C) | Ramp (s) | Hold (s) | Ar flow rate (mL $min^{-1}$) |
|---|---|---|---|---|
| **Drying** | 85 | 5 | 0 | 300 |
| | 95 | 40 | 0 | 300 |
| | 120 | 10 | 0 | 300 |
| **Pyrolysis**[a] | 1400 (Cr) | 5 | 4 | 300 |
| | 1000 (Mn) | | | |
| **Atomization**[a] | 2600 (Cr) | 1 | 3 | 0 |
| | 1800 (Mn) | | | |
| **Cleaning** | 2700 (Cr) | 0.5 | 2 | 300 |
| | 1900 (Mn) | | | |

[a] Optimum temperatures of pyrolysis and atomization were established through the construction of the respective curves (see text for details).

The decontamination was carried out by soaking the flasks in a 10% v/v $HNO_3$ solution for 24 h, at least, followed by rinsing with purified water. Then, they were dried at ambient temperature in a dust free environment and stored in a clean place until their use.

## 3. Results and discussion

As mentioned previously, this work proposes a new methodology for the determination of trace concentrations of Cr and Mn in edible oils. The optimization of the methodology took into account the magnitude of the analytical signals for each metal and the time required to break the emulsions. While the analytical signals expressed the efficiency achieved in the extraction, the time required to break the emulsions gave a good estimative of the time spent to complete the procedure.

### 3.1. Construction of the curves of pyrolysis and atomization

The first part of the experimental work was to establish the correct program of temperature for the measurement of Cr and Mn in the extracts by ETAAS. This study was performed through the construction of the curves of pyrolysis and atomization for each metal. The temperature of the drying step was set taking into consideration only the elimination of water because the extracts obtained in the extraction induced by emulsion breaking were aqueous. The drying step had a maximum temperature of 120 °C, which was enough to ensure total water vaporization [29]. The Table 2 shows the complete temperature programs.

Two curves of pyrolysis and atomization were constructed for Cr and Mn; one using aqueous standard solutions (10 µg $L^{-1}$ of each metal) and other using the extract obtained after the application of the proposed procedure to the reference sample. The extraction induced by emulsion breaking was carried out with 5 mL of vegetable oil that were emulsified with 1 mL of a 5% m/v Triton X-114 solution prepared in 1.4 mol $L^{-1}$ $HNO_3$ medium. The emulsion was broken at 80 °C and the extract was injected into the graphite tube.

The main goal of this experiment was to compare the thermal behavior of the analytes when they were in the aqueous solution and in the extract. The comparison between the two profiles allowed that possible interferences on the Cr and Mn signals could be predicted due to the presence of any component of the extractant solution or due to any substance transferred from the oil to the extract during the extraction. These substances could increase the background signal and/or enhance the formation of volatile species of Cr and Mn. With the information regarding the thermal behavior of the analytes in the extract, it was possible to select correct pyrolysis and atomization temperatures for the measurements.

**Table 2**
Limits of detection reported in the literature for the determination of Cr and Mn in vegetable oils by different methods.

| Reference | Treatment of sample | Analytical technique | Limit of detection |
|---|---|---|---|
| **Cindric et al.** [12] | Total dissolution | ICP OES | Cr (0.81 $\mu g\ g^{-1}$)<br>Mn (0.08 $\mu g\ g^{-1}$) |
| | | GFAAS | Cr (0.001 $\mu g\ g^{-1}$)<br>Mn (0.005 $\mu g\ g^{-1}$) |
| **Benincasa et al.** [13] | Total dissolution | ICP-MS | Cr (16.3 $ng\ g^{-1}$)[a]<br>Mn (9.2 $ng\ g^{-1}$)[a] |
| **Zeiner et al.** [31] | Total dissolution | ETAAS | Cr (1 $ng\ g^{-1}$)<br>Mn (1 $ng\ g^{-1}$) |
| **Bakkali et al.** [32] | Total dissolution | GFAAS | Cr (1.30 $mg\ kg^{-1}$)<br>Mn (0.14 $mg\ kg^{-1}$) |
| **Juranovic et al.** [10] | Total dissolution | ICP OES | Cr (0.880 $\mu g\ g^{-1}$)<br>Mn (0.032 $\mu g\ g^{-1}$) |
| **Martin-Polvillo et al.** [33] | Direct injection of the samples | GFAAS | Cr (6.8 ppb by weight) |
| **Fischer and Rademeyer** [34] | Direct injection of the samples | ICP OES | Cr (0.051 $\mu g\ g^{-1}$) |
| **Karadjova et al.** [4] | Sample dilution in solvent | ETAAS | Cr (0.02 $\mu g\ g^{-1}$)<br>Mn (0.01 $\mu g\ g^{-1}$) |
| **Marfil et al.** [7] | Sample dilution in solvent | ETAAS | Cr (1 pg)[b]<br>Mn (2 pg)[b] |
| **de Souza et al.** [20] | Injection of microemulsion | ICP OES | Cr (2.8 $ng\ g^{-1}$)<br>Mn (0.9 $ng\ g^{-1}$) |
| **Jiménez et al.** [34] | Injection of emulsion | ICP-MS | Mn (0.98 $mg\ kg^{-1}$) |
| **Castillo et al.** [2] | Injection of emulsion | ICP-MS | Cr (50 $ng\ g^{-1}$)<br>Mn (3.5 $ng\ g^{-1}$) |
| **This work** | Extraction induced by emulsion breaking | ETAAS | Cr (66 $ng\ L^{-1}$)<br>Mn (36 $ng\ L^{-1}$) |

[a] The article only reported the limit of quantification.
[b] The volume of sample injected was not specified.

The Fig. 2 shows the curves of pyrolysis and atomization for Cr and Mn. The injection of the aqueous standards or the extract into the graphite tube provided curves of pyrolysis and atomization with very similar profile, indicating that the analytes presented the same thermal behavior when they were in the extract or in the aqueous standard solution. The background signals observed in the measurement of the extract were very low even when a pyrolysis temperature as low as 500 °C was tested, evidencing that the matrix could be easily eliminated in the pyrolysis step.

Based on the temperature profiles obtained in this study, the temperatures of pyrolysis for Cr and Mn were set at 1400 and 1000 °C, respectively, while the temperatures of atomization were 2600 and 1800 °C, respectively. Chemical modifiers were not added because there was no evidence of the volatilization of the analytes in the pyrolysis step, which could decrease the sensitivity. Moreover, the addition of any chemical modifier could contaminate the blanks and impair the limit of detection and quantification.

The application of the final program of temperature optimized in this work in the measurement of Cr and Mn in the extracts of the reference sample generated well-defined atomic absorption peaks with low background signals, which were easily corrected by the Zeeman effect-based corrector, as shown in the Fig. 3.

### 3.2. Effect of the concentration of $HNO_3$

The metals can be present in the edible oils in the free form, in the organic molecules or as part of organic complexes. The acid added to the emulsions displaces the metals of the organic structures, making possible their transference to the aqueous phase as free ions [26]. Because of this phenomenon, the concentration of the acid added to the aqueous solution used to prepare the emulsions should be evaluated.

In this first extraction experiment, the initial conditions were those previously proposed by our group for the extraction of metals from diesel oil: the concentration of the emulsifier agent was 4% m/v (for both Triton X-100 and Triton X-114) and the temperature for emulsion breaking was 80 °C [26]. The concentration of $HNO_3$ in the solution was varied in the range of 0.28–5.6 mol $L^{-1}$. The Fig. 4 shows the variation of the analytical signals with the variation of the concentration of $HNO_3$ for Cr and Mn.

The analysis of the results showed that the concentration of $HNO_3$ in the aqueous solution did not influence significantly the extraction efficiency, except for Cr when the Triton X-114 was used as emulsifier agent. In this case, the signals increased with the increase of the concentration of $HNO_3$ until 2.8 mol $L^{-1}$. For concentrations higher than 2.8 mol $L^{-1}$, the signals remained constant. This fact indicated that Cr could be bonded to the organic matrix through stronger interactions than Mn.

Other aspect evaluated in this experiment was the influence of the concentration of $HNO_3$ on the time required to break the emulsions. As it can be seen in the Fig. 5, the increase of the concentration of $HNO_3$ caused a noticeable decrease of the time needed to break the emulsions at 80 °C, when both Triton X-100 and X-114 were used. Obviously, other parameters such as the concentration of the emulsifier agent and the temperature should also have influence on the time needed for emulsion breaking and required further studies.

Another point taken into account was the acidity of the final extract obtained after application of the extraction induced by emulsion breaking, since the acidity of the solution injected into instrument has remarkable influence on the number of firings of the graphite tubes. The number of firings was increased with the injection of the extract with lowest acidity. So, in order to achieve a convenient extraction for both analytes, save time and increase the number of firings of the graphite tubes, without losing extraction efficiency, the concentration of $HNO_3$ selected for the method was 2.8 mol $L^{-1}$.

### 3.3. Effect of the concentration and structure of the emulsifier agent

The addition of the emulsifier agent is a fundamental part of the extraction induced by emulsion breaking procedure because it allows that water droplets containing the acid could be efficiently

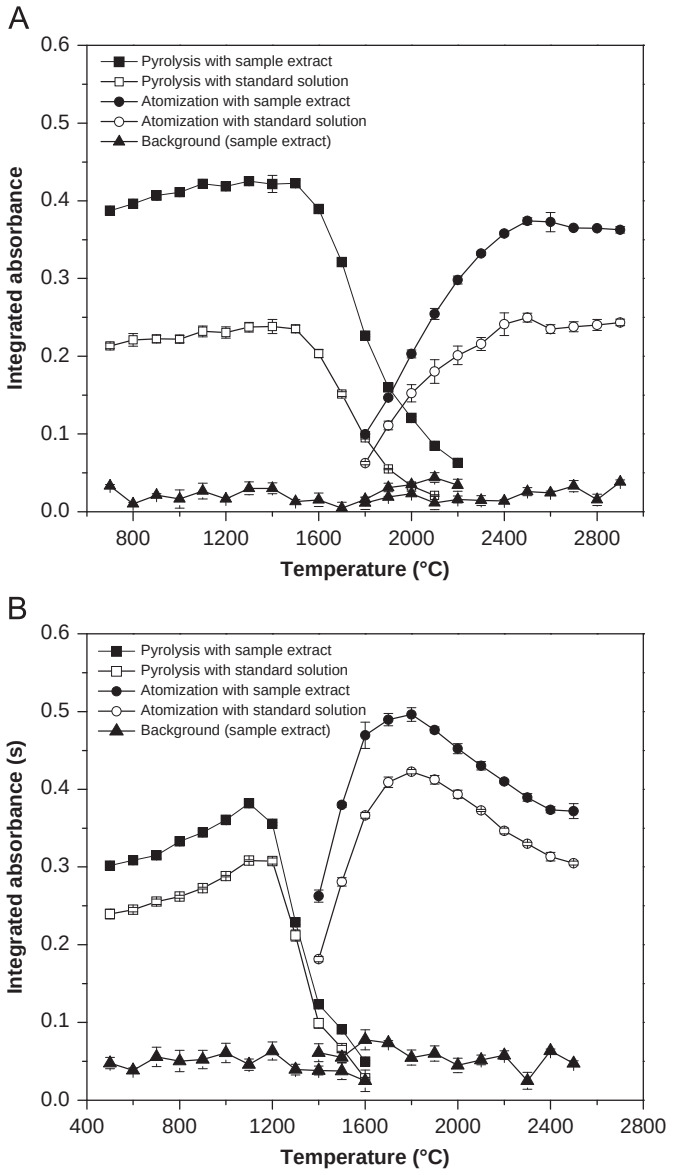


**Fig. 2.** Curves of pyrolysis and atomization for Cr and Mn in the aqueous standard medium (10 μg $L^{-1}$ of each metal) and in the extract obtained by the proposed method (see text for details). (A) Cr and (B) Mn.

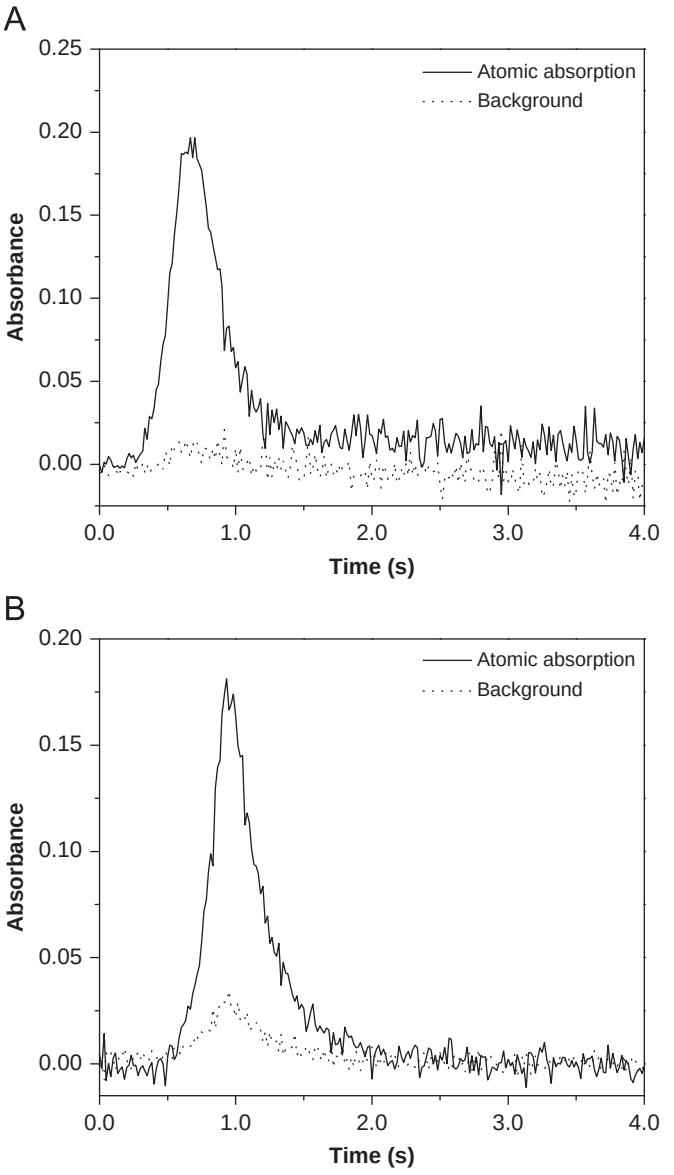


**Fig. 3.** Profiles of the peaks of (A) Cr and (B) Mn obtained in the injection of the extract obtained by the proposed method (see text for details), using the optimized program of temperature.

dispersed through the oil, forming the emulsion. This process promotes a more intense contact between the oil and the acid solution used for the extraction and enhances the extraction efficiency.

The effect of the emulsifier agent was evaluated taking into consideration two aspects: (A) its concentration in the solution used for emulsification and (B) the structure of the emulsifier agent (Triton X-100 or Triton X-114). Initially, the influence of the concentration of the emulsifier agent on the extraction efficiency was investigated for both surfactants. The extraction was carried out with 5 mL of the reference sample and 1 mL of the aqueous solution containing the surfactant and $HNO_3$ (2.8 mol $L^{-1}$). The concentration of Triton X-100 and X-114 was varied from the minimum required to form the emulsion, which was 0.5% m/v, to 20% m/v. The Fig. 6 shows the results obtained in the experiment.

The increase of the concentration of the emulsifier agents increased the extraction efficiency, which was denoted by the increase of the analytical signals for Cr and Mn. Curves with similar profiles were verified when Triton X-100 and X-114 were employed for the emulsification, indicating that an efficient extraction of the metals can be reached independently of the emulsifier agent. These results also showed that the increase of the concentration of the emulsifier agent leaded to the formation of more stable emulsions, with smaller water droplets, which caused more intense contact between the aqueous (extractant) and organic phases (sample). As result, the extraction efficiency was enhanced.

Although the extraction efficiency increased, the formation of more stable emulsions also increased the time needed to their breaking, which was undesirable. As example, when the concentration of Triton X-100 or X-114 was 15% m/v (region of maximum extraction efficiency for Cr and Mn), the heating time at 80 °C for emulsion breaking was longer than 60 min for both surfactants.

### 3.4. Influence of temperature on the emulsion breaking

The simplest way to solve the problem verified in the study of the concentration of the emulsifier (high time for emulsion

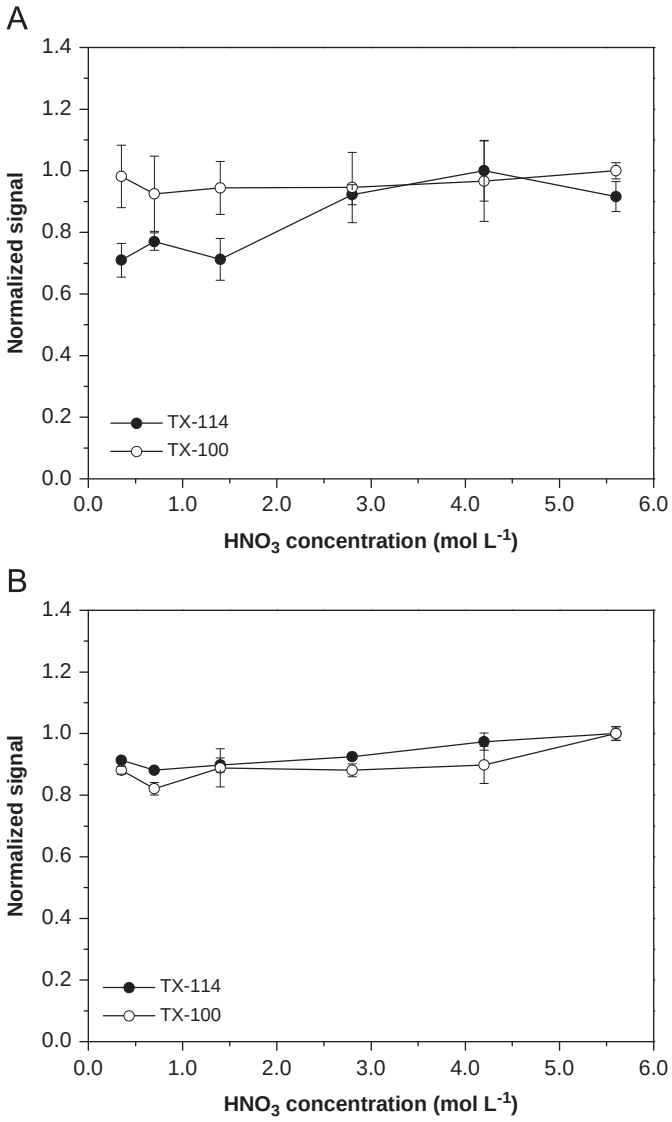


**Fig. 4.** Influence of the concentration of the $HNO_3$ on the extraction of (A) Cr and (B) Mn by the proposed method. Triton (X-100 or X-114) concentration = 4% m/v.

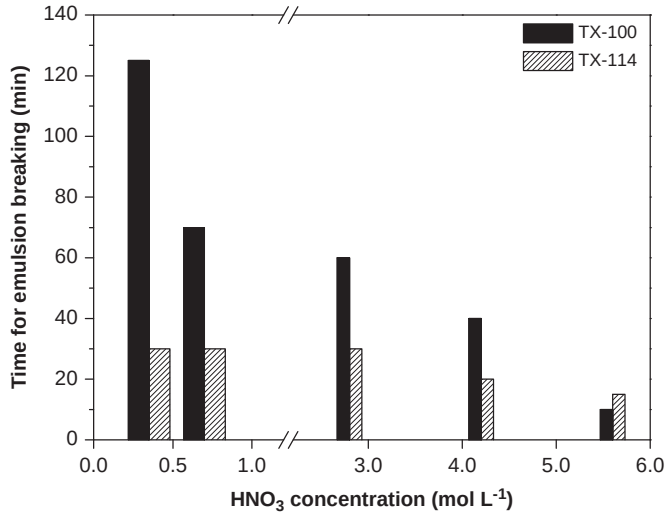


**Fig. 5.** Influence of the concentration of $HNO_3$ on the time needed for emulsion breaking. Triton (X-100 and X-114) concentration = 4% m/v and temperature of the water bath = 80 °C.

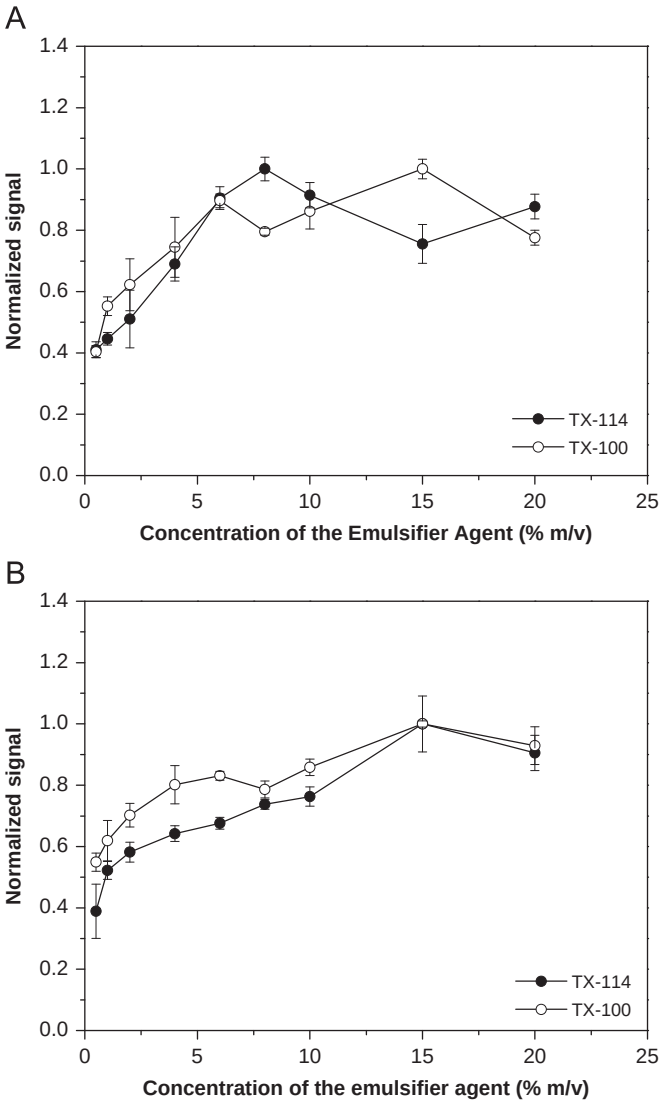


**Fig. 6.** Influence of the concentration of the emulsifier agent on the extraction of metals by the proposed method. (A) Triton X-100 and (B) Triton X-114. $HNO_3$ concentration = 2.8 mol $L^{-1}$ and temperature of the water bath = 80 °C.

breaking) was to change the temperature of heating. The emulsions are broken by increasing the temperature because the viscosity of the oil decreases, allowing that the water droplets move through the emulsion and aggregate. The aggregation of the water results in the formation of bigger droplets, which coalesce [30].

Firstly, the influence of the temperature applied for the emulsion breaking on the extraction efficiency of Cr and Mn was tested in the range of 70–90 °C using both Triton X-100 and Triton X-114 as emulsifier agents. The concentration of the emulsifier agents in the experiments was 15% m/v. No variation of the analytical signals was noted for Cr and Mn, evidencing that the extraction efficiency did not depend on the temperature employed for the emulsion breaking. On the other hand, the time required to break the emulsions was strongly affected by the temperature. While at 70 °C the emulsions were broken after more than 3 h of heating, for both Triton X-100 and Triton X-114, at 90 °C, only 10 min were needed to promote the total separation of the aqueous and organic phases when Triton X-114 was used. Also, for Triton X-100 at 90 °C, the time required for emulsion breaking was 30 min (Fig. 7). Similar behavior was previously verified for the emulsion breaking of water-in-oil emulsions prepared with diesel oil [26].

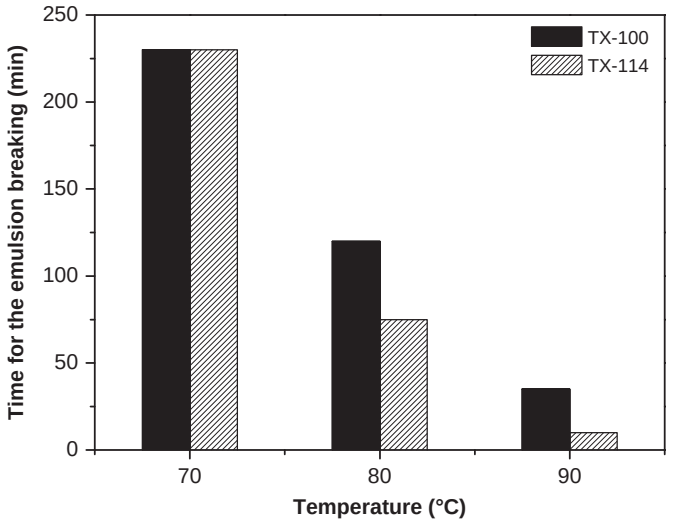


**Fig. 7.** Effect of the temperature employed on the time needed to achieve the emulsion breaking. Triton (X-100 or X-114)=15% m/v and $HNO_3$=2.8 mol $L^{-1}$.

In front of the results obtained, the temperature of 90 °C was selected for the emulsion breaking in all further experiments and the use of Triton X-100 was avoided because of the higher time needed to achieve the emulsion breaking when using this surfactant for the preparation of the emulsions. So, Triton X-114 (15% m/v) was chosen as emulsifier agent for the method.

### 3.5. Effect of the collection time

The process of emulsion breaking generates two immiscible phases and, consequently, an interfacial film between them, where the analytes could be adsorbed. The adsorption of Cr and Mn ions on the interface could result in the decrease of the extraction efficiency, especially if the phases are kept in contact for long times. This phenomenon occurs because the analytes, if adsorbed, are not collected when the aqueous phase is taken out for the measurements. So, in order to verify the occurrence (or not) of this problem, an experiment was performed by varying the time elapsed between the emulsion breaking and the collection of the aqueous phase, defined as the collection time. Two different experimental conditions were tested: (A) keeping the broken emulsion in the water bath at 90 °C and (B) taking the flask with the two phases out of the water bath just after the emulsion was broken. The collection time was varied from 2 min, which represents the minimum time for the separation of the phases with the micropipette, to 120 min in both experiments.

The variation of the signals for Cr and Mn in the two situations and with the time was negligible, evidencing that the analytes do not tend to migrate from the bulk of the solution to the interface when the aqueous and organic phases are kept in contact for 120 min, at least.

## 4. Evaluation of the calibration strategy and analytical features

The use of a correct approach for calibration is essential for the success of any analytical methodology. Therefore, it should be studied carefully in order to avoid systematic errors in the procedure. One of the main advantages of the proposed method is that the extraction induced by emulsion breaking allows the transference of the analytes from the complex organic matrix to a simple aqueous phase, making possible that the calibration could be performed with aqueous standard solutions [26]. However, the surfactant used as emulsifier agent to prepare the emulsions could also be transferred to the aqueous phase during the emulsion breaking because of its high solubility in water. The presence of the surfactant in the aqueous extracts could alter their physical characteristics, which could result in the occurrence of matrix interferences on the method. In order to test if the extracts were similar (or not) to the aqueous standards, standard-addition curves were constructed and compared with analytical curves constructed with simple aqueous standards. For Cr, the slopes of the analytical and standard-addition curves were $0.0232 \pm 0.0004$ and $0.0244 \pm 0.0010$, respectively, and for Mn, the slopes were $0.0415 \pm 0.0004$ and $0.0413 \pm 0.0002$, respectively. The application of the Student-t test (95% confidence level) showed that there was no statistical difference between the slopes for both metals, proving that the extracts can be analyzed using analytical curves prepared with aqueous standard solutions.

Once it was verified that matrix interferences were not significant, the limits of detection and quantification were derived for the method using the analytical curves prepared in purified water. The limits of detection ($3\sigma$ criterion) and quantification ($10\sigma$ criterion) were estimated from ten measurements of the blank solutions, which were extracts obtained from the application of the extraction induced by emulsion breaking to a soybean oil sample containing Cr and Mn in concentrations that were not detectable by the proposed method. The limits of detection for the measurement of Cr and Mn in the extracts were 0.33 and 0.18 μg $L^{-1}$, respectively, and the limits of quantification were 1.1 and 0.60 μg $L^{-1}$, respectively. Such limits could also be derived for the determination of the metals of interest in the original samples, taking into account the preconcentration factor obtained by the application of the proposed extraction procedure that was 5 (5 mL of edible oil to 1 mL of extractant solution). In this case, the limits of detection for Cr and Mn were 66 and 36 ng $L^{-1}$, respectively, and the limits of quantification were 219 and 120 ng $L^{-1}$, respectively. As shown in the Table 2, the limits of detection obtained in this work were lower than those reported in other studies for the determination of Cr and Mn in vegetable oils when different treatment of samples and analytical techniques were employed [2,4,7,10,12,13,20,31–35]. The precision was estimated from the application of the proposed method in the determination of Cr and Mn in ten independent aliquots of the sample $S_8$. Relative standard deviations of 7.5% and 8.4% were verified for Cr and Mn, respectively.

## 5. Application of the proposed methodology

The evaluation of the accuracy of the proposed procedure was assessed by performing the recovery test with twelve samples of edible oils with different origins, which is the strategy widely employed for this kind of sample [36]. The verification of the accuracy of the method through the analysis of certified reference materials could not be applied, since there are no certified reference materials of edible oils for metals. Besides, the application of other analytical methodologies was not viable, since they required the digestion of the samples, which leaded to the high dilution of the samples and made impossible the quantification of the analytes in the obtained solutions.

The recovery test for Cr was done with the addition of known concentrations of the metal in the form of an organometallic standard diluted in hexane. Although the Cr added as organometallic standard was different of the Cr originally present in the sample, the recovery test employing this standard was the unique alternative to test the accuracy of the procedure. The analyte was added to the samples in two concentrations: 2.0 and 4.0 μg $L^{-1}$.

In the case of Mn, the recovery test was performed by two approaches. The first was the same used for Cr, i.e., the Mn was added to the samples as an organometallic standard diluted in hexane. Again, two concentrations of addition were tested: 4.0 and 8.0 μg $L^{-1}$. Secondly, the sample of olive oil containing a known concentration of Mn (112 ± 17 μg $L^{-1}$) was added to the samples $S_1$-$S_6$ in a proportion of 5% v/v in order to generate a concentration of 5.6 μg $L^{-1}$ of the metal. As mentioned previously, the concentration of Mn in the olive oil was determined by ETAAS after its total digestion in a closed-vessel microwave oven.

The Tables 3 and 4 show the results obtained in the recovery test for Cr and Mn, respectively. Recovery ratios in the range of 89–115% and 86–115% were verified for Cr and Mn, respectively. Such results attested the efficiency of the extraction procedure proposed in this work in removing the metals from the edible oils to the aqueous phase, independently of the nature of the oil.

Additionally, the concentration of Mn in the reference sample was determined by the proposed method in three consecutive days and compared with the concentration of Mn determined by ETAAS after the total digestion of the sample (olive oil). The results obtained in this experiment are shown in the Table 5. There was no statistical difference between the results when the Student-t test (95% confidence level) was applied, which proved the accuracy of the proposed method for Mn determination in the edible oils. The intermediary precision was calculated from the standard deviation of the mean obtained after the determination of Mn in the three consecutive days. The intermediary precision was 5.0%.

## 6. Conclusions

The application of the extraction induced by emulsion breaking in the determination of Cr and Mn in edible oils was studied in this present work. The optimized method showed to be adequate for the extraction of the metals under study from oils of different origin (soybean, corn, sunflower canola and olive oil), allowing their determination by ETAAS without interference of the matrix of the samples that contain high carbon content.

Once the analytes were efficiently extracted to an aqueous extractant solution (2.8 mol $L^{-1}$ $HNO_3$ + 15% m/v Triton X-114), the external calibration approach could be applied, which simplified the experimental procedure. Besides, a preconcentration factor of 5 was achieved, which permitted the determination of Cr and Mn at ng $L^{-1}$ level. The total time for the pretreatment of the samples was approximately 15 min and was lower than the time spent in the classical acid digestion procedures regularly employed for the mineralization of the samples.

The application of the extraction induced by emulsion breaking to the analysis of edible oils presented similar characteristics in comparison with the analysis of diesel oil by the same extraction approach [26–28]. The time needed to break the emulsions prepared with either diesel or edible oils were practically the same, indicating that the stability of the emulsions were very similar. The main difference was the appearance of black residues of oxidized oil in the extraction of diesel oil, which were not observed in the case of edible oil, which resulted in a cleaner extract. Nevertheless, the metals could be easily quantified in the

**Table 3**
Results obtained in the determination of Cr in the samples of edible oils by the developed method. Values are expressed as mean ± standard deviation ($n$=3).

| Sample | Type of oil | Cr concentration (μg $L^{-1}$) | Recovery test (%) | |
|---|---|---|---|---|
| | | | 2.0 μg $L^{-1}$ (oil standard) | 4.0 μg $L^{-1}$ (oil standard) |
| $S_1$ | Soybean | < LQ | 109 ± 1 | 106 ± 2 |
| $S_2$ | Soybean | < LQ | 90 ± 3 | 93 ± 10 |
| $S_3$ | Sunflower | < LQ | 109 ± 9 | 114 ± 4 |
| $S_4$ | Canola | 0.29 ± 0.04 | 89 ± 5 | 91 ± 4 |
| $S_5$ | Olive + Soybean | 1.76 ± 0.05 | 105 ± 8 | 101 ± 5 |
| $S_6$ | Olive + Soybean | 5.81 ± 0.83 | 102 ± 6 | 115 ± 1 |
| $S_7$ | Olive + Soybean | 1.39 ± 0.02 | 107 ± 7 | 108 ± 4 |
| $S_8$ | Olive + Soybean | 2.39 ± 0.16 | 115 ± 2 | 115 ± 7 |
| $S_9$ | Soybean | 0.44 ± 0.01 | 98 ± 7 | 109 ± 10 |
| $S_{10}$ | Sunflower | 0.27 ± 0.01 | 100 ± 5 | 100 ± 2 |
| $S_{11}$ | Canola | 0.34 ± 0.06 | 106 ± 3 | 101 ± 5 |
| $S_{12}$ | Corn | 1.06 ± 0.06 | 93 ± 5 | 90 ± 2 |

**Table 5**
Results obtained in the determination of Mn in the olive oil employed in the preparation of the reference sample. Values are expressed as mean ± standard deviation ($n$=3).

| Sample | Type of oil | Mn concentration determined after total digestion (μg $L^{-1}$) | Mn concentration determined by the proposed method (μg $L^{-1}$)[b] | Intermediary precision (%) |
|---|---|---|---|---|
| $S_{13}$[a] | Olive | 112 ± 17 | 112 ± 5<br>103 ± 8<br>113 ± 3 | 5.0 |

[a] Sample $S_{13}$ was the olive oil used in the preparation of the reference sample.
[b] The determinations of the concentration of Mn by the proposed method were performed in three consecutive days (one at each day).

**Table 4**
Results obtained in the determination of Mn in the samples of edible oils by the developed method. Values are expressed as mean ± standard deviation ($n$=3).

| Sample | Type of oil | Mn concentration (μg $L^{-1}$) | Recovery test (%) | | |
|---|---|---|---|---|---|
| | | | 4.0 μg $L^{-1}$ (oil standard) | 8.0 μg $L^{-1}$ (oil standard) | 5.6 μg $L^{-1}$ (olive oil) |
| $S_1$ | Soybean | 0.37 ± 0.05 | 102 ± 3 | 103 ± 3 | 101 ± 1 |
| $S_2$ | Soybean | 0.21 ± 0.03 | 106 ± 15 | 97 ± 5 | 98 ± 14 |
| $S_3$ | Sunflower | < LQ | 108 ± 7 | 108 ± 4 | 100 ± 6 |
| $S_4$ | Canola | < LQ | 104 ± 2 | 103 ± 9 | 86 ± 3 |
| $S_5$ | Olive + Soybean | 4.22 ± 0.13 | 104 ± 5 | 92 ± 2 | 109 ± 2 |
| $S_6$ | Olive + Soybean | 0.89 ± 0.06 | 111 ± 2 | 115 ± 1 | 92 ± 1 |
| $S_7$ | Olive + Soybean | 0.19 ± 0.02 | 106 ± 2 | 113 ± 5 | - |
| $S_8$ | Olive + Soybean | 2.93 ± 0.12 | 99 ± 8 | 110 ± 4 | - |
| $S_9$ | Soybean | 3.99 ± 0.46 | 94 ± 6 | 90 ± 3 | - |
| $S_{10}$ | Sunflower | 1.42 ± 0.09 | 108 ± 8 | 98 ± 2 | - |
| $S_{11}$ | Canola | 0.82 ± 0.07 | 105 ± 4 | 100 ± 4 | - |
| $S_{12}$ | Corn | 8.62 ± 0.46 | 99 ± 10 | 89 ± 5 | - |

extracts by ETAAS or other analytical technique independently of the presence or not of the residue.

## Acknowledgments

The authors are grateful to CNPq (Conselho Nacional de Desenvolvimento Cientifico e Tecnológico), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro) for providing grants and fellowships and for financial support.

## References

[1] H.D. Belitz, W. Grosch, P. Schieberle, Food Chemistry, 4th edition, Springer-Verlag, Berlin, Germany, 2009.
[2] J.R. Castillo, M.S. Jiménez, L. Ebdon, J. Anal. At. Spectrom. 14 (1999) 1515–1518.
[3] M.N.M. Reyes, R.C. Campos, Talanta 70 (2006) 929–932.
[4] I. Karadjova, G. Zachariadis, G. Boskou, J. Stratis, J. Anal. At. Spectrom. 13 (1998) 201–204.
[5] S.S. Chen, C.M. Chen, C.C. Cheng, S.S. Chou, J. Food Drug Anal. 7 (1999) 207–214.
[6] C.M. Canário, D.M. Katskov, J. Anal. At. Spectrom. 20 (2005) 1386–1388.
[7] R. Marfil, C. Cabrera-Vique, R. Giménez, P.R. Bouzas, O. Martinez, J.A. Sánchez, J. Agr. Food Chem. 56 (2008) 7279–7284.
[8] S.E. Raptis, G. Kaiser, G. Tölg, Anal. Chim. Acta 138 (1982) 93–101.
[9] M.I. Saleh, R.S. Murray, C.N. Chin, J. Am. Oil Chem. Soc. 65 (1988) 1767–1770.
[10] I. Juranovic, P. Breinhoelder, I. Steffan, J. Anal. At. Spectrom. 18 (2003) 54–58.
[11] A. Gonzálvez, M.E. Ghanjaoui, M. El Rhazi, M. de la Guardia, Food Sci. Tech. Int. 16 (2010) 65–71.
[12] I.J. Cindric, M. Zeiner, I. Steffan, Microchem. J. 85 (2007) 136–139.
[13] C. Benincasa, J. Lewis, E. Perri, G. Sindona, A. Tagarelli, Anal. Chim. Acta 585 (2007) 366–370.
[14] L.B. Allen, P.H. Siitonen, H.C. Thompson, J. Am. Oil Chem. Soc. 75 (1998) 477–481.
[15] D. Mendil, O.D. Oluozlu, M. Tuzen, M. Soylak, J. Hazard. Mater. 165 (2009) 724–728.
[16] E. Oliveira, J. Braz. Chem. Soc. 14 (2003) 174–182.
[17] M.S. Jiménez, R. Velarte, M.T. Gomez, J.R. Castillo, At. Spectrosc. 25 (2004) 1–12.
[18] A. Anthemidis, V. Arvanitidis, J.A. Stratis, Anal. Chim. Acta. 537 (2005) 271–278.
[19] Y.T. Chang, S.J. Jiang, J. Anal. At. Spectrom. 23 (2008) 140–144.
[20] R.M. de Souza, B.M. Mathias, C.L.P. da Silveira, R.Q. Aucélio, Spectrochim. Acta Part B 60 (2005) 711–715.
[21] L.S. Nunes, J.T.P. Barbosa, A.P. Fernandes, V.A. Lemos, W.N.L. dos Santos, M.G.A. Korn, L.S.G. Teixeira, Food Chem. 127 (2011) 780–783.
[22] A. Leonardis, V. Macciola, M. Felice, Int. J. Food Sci. Technol. 35 (2000) 371–375.
[23] E. Pehlivan, G. Arslan, F. Gode, T. Altun, M.M. Özcan, Grasas y Aceites 59 (2008) 239–244.
[24] F. Anwar, T.G. Kazi, R. Saleem, M.I. Bhanger, Grasas y Aceites 55 (2004) 160–168.
[25] R. Ooms, W.V. Pee, J. Am. Oil Chem. Soc. 60 (1983) 957–960.
[26] R.J. Cassella, D.M. Brum, C.E.R. de Paula, C.F. Lima, J. Anal. At. Spectrom. 25 (2010) 1704–1711.
[27] R.J. Cassella, D.M. Brum, C.F. Lima, L.F.S. Caldas, C.E.R. de Paula, Anal. Chim. Acta 690 (2011) 79–85.
[28] R.J. Cassella, D.M. Brum, N.F. Robaina, A.A. Rocha, C.F. Lima, J. Anal. At. Spectrom. 27 (2012) 364–370.
[29] D.J. Butcher, J. Sneddon, A practical guide to graphite furnace atomic absorption spectrometry, John Wiley & Sons, New York, USA, 1998.
[30] L.L. Schramm (Ed.), Emulsions: Fundamentals and Applications in the Petroleum Industry, American Chemical Society, Washington D.C., 1992.
[31] M. Zeiner, I. Steffan, I.J. Cindric, Microchem. J. 81 (2005) 171–176.
[32] K. Bakkali, E. Ballesteros, B. Souhail, N.R. Martos, Grasas y Aceites 60 (2009) 490–497.
[33] M. Martin-Polvillo, T. Albi, A. Guinda, J. Am. Oil Chem. Soc. 71 (1994) 347–353.
[34] J.L. Fischer, C.J. Rademeyer, J. Anal. At. Spectrom. 9 (1994) 623–628.
[35] M.S. Jiménez, R. Velarte, J.R. Castilho, J. Anal. At. Spectrom. 18 (2003) 1154–1162.
[36] F.G. Lepri, E.S. Chaves, M.A. Vieira, A.S. Ribeiro, A.J. Curtius, L.C. Oliveira, Appl. Spectrosc. Rev. 46 (2011) 175–206.