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Analytical Methods

Multivariate optimization of ultrasound-assisted extraction for determination of Cu, Fe, Ni and Zn in vegetable oils by high-resolution continuum source atomic absorption spectrometry

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ABSTRACT

An assisted liquid–liquid extraction of copper, iron, nickel and zinc from vegetable oil samples with subsequent determination by high-resolution continuum source flame atomic absorption spectrometry (HR-CS FAAS) was optimized by applying a full factorial design in two levels and the response surface methodology, Box–Behnken. The effects of the acid concentration and the amplitude, cycle and time of sonication on the extraction of the analytes, as well as their interactions, were assessed. In the selected condition (sonication amplitude = 66%, sonication time = 79 s, sonication cycle = 74%), using 0.5 mol L⁻¹ HCl as the extractant, the limits of quantification were 0.14, 0.20, 0.21 and 0.04 µg g⁻¹ for Cu, Fe, Ni and Zn, respectively, with R.S.D. ranging from 1.4% to 3.6%. The proposed method was applied for the determination of the analytes in soybean, canola and sunflower oils.

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1. Introduction

Vegetable oils are widely used for cooking in households and in the food industries. There are also several applications for vegetable oils in the cosmetic, pharmaceutical and chemical industries (Dugo, La Pera, La Torre, & Giuffrida, 2004; Zhu, Fan, Wang, Qu, & Yao, 2011). These oils are usually extracted from the seeds of cereals and legumes, such as soybean, sunflower, canola, peanuts and cotton. It is also possible to extract them from fruit, such as palm and olives.

Vegetable oils in the human diet play an important role in the absorption of fat-soluble vitamins such as A, D, E and K. They are associated with the production of satiety signals that regulate meal size, and their controlled and regular ingestion may be associated with the prevention of stress, cardiovascular and brain problems (Denis, Potier, Vancassel, Heberden, & Lavielle, 2013). Furthermore, the presence of inorganic species, such as some metal ions, has nutritional importance (Zhu et al., 2011).

However, the presence of certain metal species, such as Cu, Fe, Ni and Zn in edible vegetable oils, even at low concentrations, may

catalyze reactions that promote an increased rate of oxidation and degradation of the product, impairing the quality. These effects influence the taste and nutritional characteristics and reduce the shelf-life of the product (Bastos & Pereira, 2010; Choe & Min, 2006; Knothe & Dunn, 2003; Paz & Molero, 2000). Some metal species are potentially toxic for human consumption, depending on the concentration.

In general, the metallic contaminants in vegetable oils are from the soil, fertilizer and presence of highways or industries near the plantations where the raw materials are grown. Additionally, these contaminants may be introduced during the production of the oil from the processing, transport or storage equipment (Dugo et al., 2004; Garrido, Frias, Díaz, & Hardisson, 1994).

Thus, the determination of the metal content is an important criterion for assessing the quality of vegetable oils and constitutes an analytical challenge due to the high complexity of the matrix and the low levels of these analytes. Atomic spectrometric techniques are frequently used for the determination of metals in vegetable oils; however, the sample preparation is a crucial step in the analytical process. The main sample pre-treatment procedures that are used to determine the metal content in oil include ashing, complete acid digestion, preparing an emulsion, dilution with solvents, direct introduction of the sample and liquid–liquid extraction.

Wet ashing generally leads to good results in terms of accuracy, but the conditions are aggressive and can lead to the loss of volatile

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analytes. It is a time-consuming procedure and requires the use of high energy and filtration after the partial solubilization of the ash (Abdel-Rahman, 1984; Garrido et al., 1994). Digestion is conducted in the presence of concentrated mineral acids and oxidants, such as hydrogen peroxide, and can be aided by microwave. Typically, the digestion is performed under aggressive conditions, is time consuming and requires large amounts of reagents (Cindric, Zeiner, & Steffan, 2007; Llorent Martínez, Ortega Barrales, de Córdoba, Domínguez Vidal, & Ruiz Medina, 2011; Mendil, Uluözülü, Tüzen, & Soyak, 2009). The emulsion or microemulsion preparations involve the use of emulsifiers combined with solutions of inorganic acids or organic solvents to reduce the viscosity of the sample and facilitate the transfer step and nebulization. However, there are problems associated with the instability of emulsions (Bakircioglu, Kurtulus, & Yurtsever, 2013; Bakircioglu, Topraksever, & Kurtulus, 2014; de Souza, Mathias, da Silveira, & Aucelio, 2005; Nunes et al., 2011; Robaina, Brum, & Cassella, 2012; Viana, Bohrer, de Carvalho, do Nascimento, & da Rosa, 2014). The dilution of sample in organic solvents is a simple procedure; however, it is associated with the use of toxic reagents and has a reduced limit of detection (Cabrera Vique, Bouzas, & Oliveras López, 2012; Chaves, de Loos Vollebregt, Curtius, & Vanhaecke, 2011; Martin Polvillo, Albi, & Guinda, 1994). There is also the possibility for the direct introduction of the sample, particularly when using atomic absorption spectrometry with graphite furnace (GF AAS). This is a strategy that greatly reduces the sample preparation work and contamination risks; however, the background signal requires special attention because of the high load of organic material inherent to the sample (Reyes & Campos, 2006).

Another way to prepare samples of vegetable oils for the determination of inorganic species is through liquid–liquid extraction (Anwar, Kazi, Saleem, & Bhangar, 2004; Baran & Yaşar, 2012; de Leonardis, Macciola, & de Felice, 2000; Pehlivan, Arslan, Gode, Turkan, & Özcan, 2008). This strategy has the advantages of speed and ease of implementation. It can also reduce reagent consumption through the use of dilute acid solutions and the pre-concentration of the analytes. However, it appears that in many cases, the analyte recovery rates are low, thus making the procedure ineffective (Baran & Yaşar, 2012).

Improvements in the recoveries in liquid–liquid extraction can be obtained through the use of ultrasonic energy (Anwar et al., 2004; Pehlivan et al., 2008). Despite the good results that have been achieved using a combination of liquid–liquid extraction and ultrasound, there remains a need to conduct a careful optimization of the variables involved in the process. The optimization can be performed with multivariate procedures such as response surface methodology, which allows for a detailed evaluation of the influence of each variable in the system and the influence of the interactions between these variables (Bezerra, Santelli, Oliveira, Villar, & Escalera, 2008). Furthermore, the multiple response optimization can be performed using the desirability function to define a single ideal condition for the determination of more than one analyte (Bezerra, Bruns, & Ferreira, 2006; Brum, Lima, Robaina, Fonseca, & Cassella, 2011; Murphy, Tsui, & Allen, 2005).

The objective for this work was to perform a multivariate optimization and obtain a single condition for the ultrasound-assisted liquid–liquid extraction for use in the subsequent determination of Cu, Fe, Ni and Zn in edible vegetable oil samples by high-resolution continuum source flame atomic absorption spectrometry (HR-CS FAAS).

2. Experimental

2.1. Instrumentation

The extraction experiments were performed with an ultrasonic processor, Hielscher UP-400S, equipped with a sonotrode H14

made of high purity titanium (Teltow, Germany). A FANEM model EXCELSA4 centrifuge (Guarulhos, Brazil) was employed for the phase separation after extraction.

The analyte determinations were carried out with an Analytik Jena (Jena, Germany) contraAA 300 high-resolution atomic absorption spectrometer XBO 301, GLE equipped with a compact high-resolution double echelle monochromator, a charge-coupled device (CCD) array detector with a resolution of approximately 2 pm per pixel in the far ultraviolet range and a 300 W xenon short-arc lamp as the continuum radiation source. The number of pixels used by the detector was 3 (central pixel 1). All measurements were performed in triplicate at 324.754, 248.327, 232.003 and 213.867 nm for Cu, Fe, Ni and Zn, respectively. An oxidizing air/acetylene flame was used for the atomization of the samples.

A Vista simultaneous inductively coupled plasma optical emission spectrometer (Varian, Mulgrave, Australia), which was equipped with a Sturman – Master chamber, a V-Groove nebulizer and a charge coupled device (CCD) detector, served as a comparative method after the acid digestion of the oil samples. The measurements were performed under the manufacturer's recommended conditions for the power (1.3 kW), with the plasma, auxiliary and nebulizer gas flows at 15 L min⁻¹, 1.5 L min⁻¹ and 0.7 L min⁻¹, respectively.

An Ethos EZ closed-vessel microwave oven (Milestone, Sorisole, Italy), which was equipped with 10 tetrafluoromethoxy vessels with a volume of 100 mL and ceramic vessel jacket, was used for the sample digestion.

2.2. Reagents and samples

All of the reagents were analytical reagent grade and the solutions were prepared using deionized water (18 MΩ cm) obtained from a Milli-Q Plus system (Millipore, Bedford, MA, USA). The hydrochloric, nitric and acetic acids were supplied by Merck (Darmstadt, Germany). The aqueous and oil standard solutions were prepared, respectively, from the dilution of mono-elemental aqueous standards (1000 mg kg⁻¹) of Cu, Fe Ni and Zn (Merck) and a 900 μg g⁻¹ metallo-organic multi-element standard (AccuStandard Inc., New Haven, CT, USA).

The proposed method was applied to analyze commercial samples of soybean, corn and canola oils. The samples were purchased at a local market in the city of Salvador, Brazil.

2.3. Procedures

For the extraction procedure, a 50-mL sample was transferred to a beaker containing 10 mL of a dilute acid solution, and the system was sonicated with an ultrasonic probe (400 W, 24 kHz) immersed at the interfacial region between the oil and aqueous phases. The biphasic sample system was sonicated under various conditions according to the experimental design. After sonication, the sample was transferred to a centrifuge tube and subjected to centrifugation for 5 min at 3000 rpm. The aqueous phase was analyzed by HR-CS FAAS.

Calibration curves were obtained in aqueous or oil media using standards in a concentration range between 0.30 and 2.00 μg g⁻¹ for each analyte. The standards were prepared in an aqueous solution of 0.5 mol L⁻¹ HCl. The oil standards were prepared in a similar manner to the samples, with the metals added in the form of organometallic compounds. The analytical blank solutions were prepared in a similar manner to the samples and subjected to the extraction procedure.

Determinations by inductively coupled plasma optical emission spectrometry (ICP OES) after acid digestion were performed as a comparative method. The digestion procedure assisted by microwave was based on a procedure from the previous literature

(Nunes et al., 2011). Each oil sample (250 mg) was weighed into the digestion vessel, and 3.5 mL of concentrated HNO₃ was added. After 30 min, 2.0 mL of H₂O₂ (30%, m/m) was added to the vessel, and microwave digestion was performed with the following program at 35 bar: (I) 6 min to 750 W (temperature of 90 °C); (II) 4 min at 750 W (temperature of 90 °C); (III) 8 min to 1000 W (temperature of 180 °C); (IV) 15 min at 1000 W (temperature of 180 °C); and (V) ventilation of the system for 20 min. After digestion, the samples were diluted to 20.0 mL with water. Analytical blanks were prepared in each lot of samples.

2.4. Optimization

Extractions were performed using 1.0 mol L⁻¹ solutions of hydrochloric, nitric or acetic acid to examine the influence of the

Table 1
Experiments and multiple responses (MR) obtained from the full factorial design 2⁴.

Experiment ^a	Amplitude (%)	Cycle (%)	Time (s)	Concentration (mol L ⁻¹)	MR
01	20 (-1)	20 (-1)	30 (-1)	0.2 (-1)	0.35
02	80 (1)	20 (-1)	30 (-1)	0.2 (-1)	0.50
03	20 (-1)	80 (1)	30 (-1)	0.2 (-1)	0.69
04	80 (1)	80 (1)	30 (-1)	0.2 (-1)	0.82
05	20 (-1)	20 (-1)	100 (1)	0.2 (-1)	0.64
06	80 (1)	20 (-1)	100 (1)	0.2 (-1)	0.68
07	20 (-1)	80 (1)	100 (1)	0.2 (-1)	0.79
08	80 (1)	80 (1)	100 (1)	0.2 (-1)	0.97
09	20 (-1)	20 (-1)	30 (-1)	2 (1)	0.40
10	80 (1)	20 (-1)	30 (-1)	2 (1)	0.50
11	20 (-1)	80 (1)	30 (-1)	2 (1)	0.71
12	80 (1)	80 (1)	30 (-1)	2 (1)	0.80
13	20 (-1)	20 (-1)	100 (1)	2 (1)	0.67
14	80 (1)	20 (-1)	100 (1)	2 (1)	0.67
15	20 (-1)	80 (1)	100 (1)	2 (1)	0.75
16	80 (1)	80 (1)	100 (1)	2 (1)	1.00
17	50 (0)	50 (0)	65 (0)	1.1 (0)	0.84
18	50 (0)	50 (0)	65 (0)	1.1 (0)	0.79
19	50 (0)	50 (0)	65 (0)	1.1 (0)	0.79
20	50 (0)	50 (0)	65 (0)	1.1 (0)	0.76

^a The volumes of the sample and extraction solution were set to 50.0 and 10.0 mL, respectively.

type of acid on the extraction efficiency. In these experiments, 50.0 mL of soybean oil spiked with 1.0 μg g⁻¹ of each analyte was placed in 10.0 mL of each acid solution. The system was sonicated for 80 s, using a sonication cycle and an amplitude equal to 50% of the maximum value for the equipment.

After defining the type of acid extractant, a full factorial design at two levels (2⁴) was developed to evaluate the effects of the sonication time, the concentration of the acid solution and the cycle and amplitude of sonication in the extraction process. The experimental levels for the amplitude and cycle for sonication were defined based on the recommendations of the equipment manufacturer. Time and the concentration of the extractant were defined by considering previous studies (Anwar et al., 2004; Baran & Yaşar, 2012; de Leonardis et al., 2000; Pehlivan et al., 2008). The factors studied and their value ranges are shown in Table 1.

The factorial design totaled 20 experiments (experiments 01–20), including four replicates at the central point. After applying the factorial design, the optimal values for each variable were determined by Box–Behnken response surface methodology. The Box–Behnken design totaled 15 experiments (experiments 21–35), including three replicates at the central point. The experiments were performed at random. The data analysis was performed using the Statistica 7.0 software. In both designs, multiple responses (MR) were obtained using a mathematical-statistical tool that is based on the use of a desirability function (*D*) (Reboucas, Santos, Pimentel, & Teixeira, 2011). This function allowed combining all of the specific responses of each analyte being studied into a single response (Eq. (1)):

$$D = \sqrt[4]{d_{\text{Cu}} \cdot d_{\text{Fe}} \cdot d_{\text{Ni}} \cdot d_{\text{Zn}}} \quad (1)$$

where *d_i* is the normalized individual response of each analyte.

3. Results and discussion

To select the extractant acid, extractions were carried out using 1.0 mol L⁻¹ solutions of hydrochloric acid, nitric acid and acetic acid, and the recoveries of the four analytes were evaluated. Lower recoveries were obtained when acetic acid was used, with recoveries ranging from 45% to 55%. The recoveries were between 65% and 75% with nitric acid. When hydrochloric acid was used as

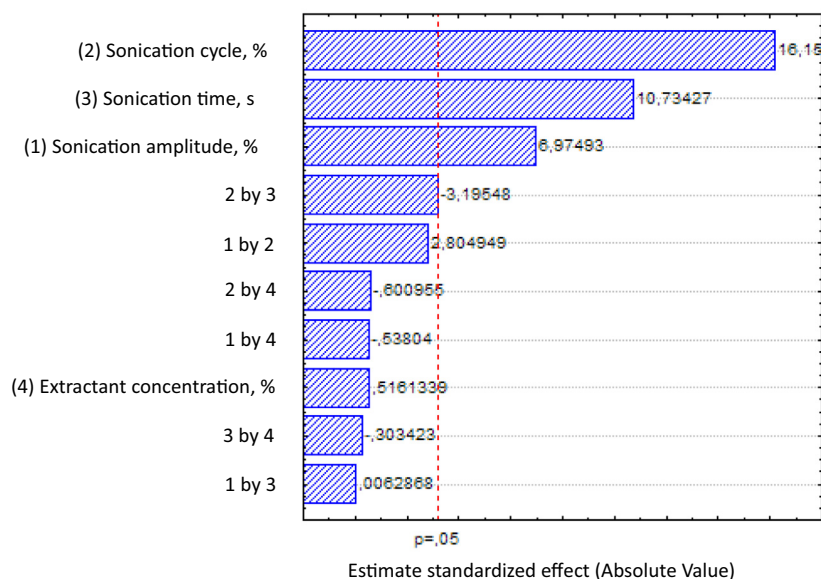


Fig. 1. Pareto chart for the full factorial design 2⁴ (95% confidence level). Experimental conditions: volumes of the sample and extraction solution were set to 50.0 and 10.0 mL, respectively.

Table 2

Experiments and multiple responses (MR) obtained from the Box–Behnken experiments.

Experiment ^a	Amplitude (%)	Cycle (%)	Time (s)	MR
21	20	20	65	0.35
22	80	20	65	0.62
23	20	80	65	0.73
24	80	80	65	1.00
25	20	50	30	0.43
26	80	50	30	0.72
27	20	50	100	0.60
28	80	50	100	0.96
29	50	20	30	0.40
30	50	80	30	0.84
31	50	20	100	0.63
32	50	80	100	0.98
33	50	50	65	0.95
34	50	50	65	0.94
35	50	50	65	0.92

^a The volumes of the sample and extraction solution (0.5 mol L⁻¹ HCl) were set to 50.0 and 10.0 mL, respectively.

the extractant, all of the recoveries for the four metals were greater than 70%. Thus, hydrochloric acid solution was selected as the extractant.

After determining the type of acid for the extraction, the effects of the extractant concentration, time, and the amplitude and cycle for sonication of the liquid–liquid extraction were evaluated. In order to obtain a higher preconcentration factor and consequently better limit of quantification, a large volume of sample and a small volume of extractant are necessary. However, the minimum sample volume for the operation of the ultrasonic probe was 50 mL. The use of larger sample volumes results in a large consumption of the sample. On the other hand the minimum volume for the multi-element determination by HR-CS FAAS was 10 mL for the aqueous solution. Then, for all experiments, the volumes of the sample and extraction solution were set to 50.0 and 10.0 mL, respectively and this ratio restricted the possibility of improving the limit of quantification. The results of the experiments obtained with the full factorial design (experiments 01–20) employing the MR are shown in Table 1. Evaluations of the effect of each variable were based on the Pareto chart shown in Fig. 1.

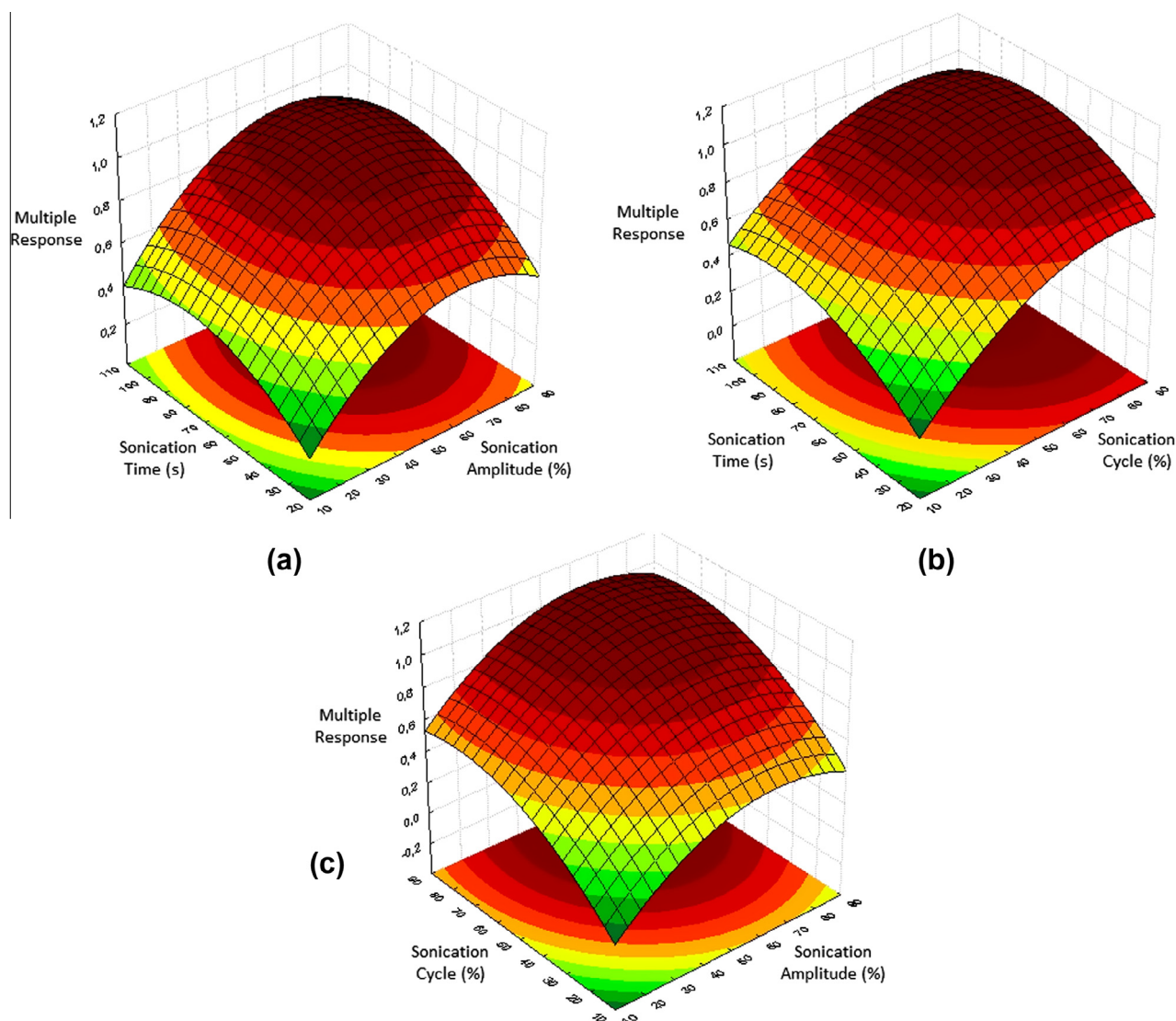


Fig. 2. Response surfaces obtained from the Box–Behnken design to the (a) amplitude and time of sonication; (b) cycle and time of sonication; (c) cycle and amplitude of sonication. Experimental conditions: volumes of the sample and extraction solution (0.5 mol L⁻¹ HCl) were set to 50.0 and 10.0 mL, respectively.

Evaluating the results presented in Fig. 1 demonstrates that the time, cycle and amplitude for sonication are significant variables for the extraction process. This result shows that ultrasonic action enhances the extraction process, favoring the transfer of analytes from the organic phase into the aqueous phase. Individually, cycle, time and amplitude of sonication were the variables that had the most significant effects. Increasing the values of these variables from the lowest (−1) to the highest (+1) level leads to an increase in the MR. This finding indicates that these three factors positively influence the transfer of analytes from the organic phase into the aqueous phase. The extractant concentration and the interactions between the variables were not found to be significant in the experimental at a confidence level of 95%.

The optimal values for each variable were determined via response surface methodology using the Box–Behnken design (experiments 21–35). The results of the Box–Behnken experiments are shown in Table 2. Once the acid concentration was determined not to be significant, the variables optimized in the Box–Behnken design were time, cycle and amplitude of sonication. The concentration of the acid extractant was fixed at 0.5 mol L^{−1}, considering that this concentration provides a good nebulization efficiency and is less aggressive on the equipment. Additionally, the experiments using 0.2 mol L^{−1} solutions did not show acceptable repeatability, probably due to the decreased concentration of chloro-complexes, since the formation and the distribution factor of these complexes can be affected by the hydrochloric acid concentration (Bernardis, Grant, & Sherrington, 2005; Karger & Miller, 1969). This fact eliminated the possibility of using the lower limit in the experimental design. The response surfaces were constructed from the data obtained using the Box–Behnken design (Fig. 2).

From the response surface, it was confirmed that the cycle, amplitude and sonication time favor the recovery of the analytes at their highest levels. The evaluation was made by the analysis of variance (ANOVA) at 95% confidence level, along with the significance levels represented by the *p*-value. It was observed that the lack of fit was not significant because the *p*-value was greater than 0.05. Thus, the model obtained is well fit to the data collected by the Box–Behnken design and can be represented by the quadratic model shown in Eq. (2):

$$\text{MR} = -0.9312 + 0.0202A + 0.0205B + 0.0146C - 0.00016A^2 - 0.00013B^2 - 0.000089C^2 \quad (2)$$

where MR represents the multiple response, *A* is the amplitude, *B* is the cycle and *C* is the time of sonication. The model showed a maximum response for the following experimental conditions (critical points): sonication amplitude = 66%, sonication time = 79 s, sonication cycle = 74%.

The model obtained was also evaluated by comparing the predicted values (*V_p*) and the experimental values (*V_e*). There was a good correlation between these values, confirming that the model fits well to the experimental data obtained from the Box–Behnken design, according to Eq. (3):

$$V_p = 0.9968 \cdot V_e + 0.0024 \quad (r = 0.9984) \quad (3)$$

Calibration curves were obtained, in a concentration range between 0.30 and 2.00 μg g^{−1} for each analyte, by using aqueous standards and via the addition of external analytes to the soybean oil sample using the extraction procedure with the optimized conditions. The curves obtained are shown in Table 3. For the equations fit to the calibration curves, the ratio of the angular coefficients is close to 1. This finding indicates that the determination of copper, iron, nickel and zinc using the proposed method for the extraction can be performed using external calibration with aqueous standards. The external calibration indicates the reliability and practicality of the method.

Table 3

Calibration curves ($A = a [C] + b$)^a obtained in aqueous and oily^b media for determination of Cu, Fe, Ni and Zn in vegetable oils.

Analyte	Medium	Calibration curves	Correlation coefficient
Copper	Aqueous	$A = 0.1003 [\text{Cu}] - 0.0022$	0.9989
	Oily	$A = 0.1027 [\text{Cu}] + 0.4565$	0.9979
Iron	Aqueous	$A = 0.0473 [\text{Fe}] - 0.0001$	0.9998
	Oily	$A = 0.0476 [\text{Fe}] + 0.4151$	0.9990
Nickel	Aqueous	$A = 0.0436 [\text{Ni}] - 0.0001$	0.9997
	Oily	$A = 0.0438 [\text{Ni}] + 0.0868$	0.9997
Zinc	Aqueous	$A = 0.2436 [\text{Zn}] - 0.0005$	0.9997
	Oily	$A = 0.2497 [\text{Zn}] + 0.1019$	0.9993

^a *A* = absorbance; *a* = slope; [*C*] = concentration (μg g^{−1}).

^b After extraction procedure. Experimental conditions: sonication amplitude = 66%, sonication time = 79 s, sonication cycle = 74%, using 0.5 mol L^{−1} HCl as the extractant.

Table 4

Determination of copper, iron, nickel and zinc (μg g^{−1}) in commercial edible vegetable oils by HR-CS FAAS after ultrasound-assisted extraction (*n* = 3).

Analyte	Oil sample	Proposed method	Comparative method
Copper	Soybean	0.83 ± 0.03	0.84 ± 0.07
	Sunflower	0.81 ± 0.01	0.82 ± 0.07
	Canola	0.81 ± 0.01	0.82 ± 0.03
Iron	Soybean	1.75 ± 0.11	1.77 ± 0.15
	Sunflower	0.96 ± 0.01	0.95 ± 0.09
	Canola	0.27 ± 0.08	0.24 ± 0.06
Nickel	Soybean	0.32 ± 0.02	0.33 ± 0.06
	Sunflower	0.24 ± 0.01	0.24 ± 0.06
	Canola	0.25 ± 0.04	0.21 ± 0.07
Zinc	Soybean	0.11 ± 0.01	0.13 ± 0.03
	Sunflower	0.14 ± 0.01	0.16 ± 0.03
	Canola	0.45 ± 0.03	0.47 ± 0.06

Experimental conditions: sonication amplitude = 66%, sonication time = 79 s, sonication cycle = 74%, using 0.5 mol L^{−1} HCl as the extractant.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as the concentration of analyte corresponding to three and ten times, respectively, the standard deviation of ten independent measurements of the analytical blank divided by the slope of the calibration curve. The LOD were 41, 61, 63 and 12 μg kg^{−1}, and the LOQ were 0.14, 0.20, 0.21 and 0.04 μg g^{−1} for Cu, Fe, Ni and Zn, respectively. The precision, estimated as the relative standard deviation (RSD), was determined from ten measurements for the extractions performed on a sample of soybean oil with 1.0 μg g^{−1} of each analyte added. The RSD were 1.4%, 2.5%, 2.0% and 3.6% for Cu, Fe, Ni and Zn, respectively.

The proposed method was applied for the determination of copper, iron, nickel and zinc in samples of commercial edible vegetable oils. The results were compared with those obtained using ICP OES after the samples were digested (Table 4). It was found that the results obtained with the proposed method are in agreement with the data obtained using the comparative method.

4. Conclusion

The proposed ultrasound-assisted procedure was efficient for the extraction of Cu, Fe, Ni and Zn from vegetable oil samples with subsequent determination by HR-CS FAAS. The multivariate optimization showed that the amplitude, cycle and time of sonication were significant variables that had a positive effect on the extraction efficiency of the analytes, indicating that the ultrasonic energy assisted the extraction process. The combination of liquid–liquid extraction aided by ultrasound with measurements by

HR-CS FAAS is a good alternative for the determination of metals in vegetable oils without the use of a time-consuming sample treatment and without the use of concentrated acids. The proposed procedure reduced reagent consumption through the use of dilute acid solutions and promoted the pre-concentration and separation of the analytes from the organic phase. The solution obtained after the extraction procedure can be analyzed directly by HR-CS FAAS without the instrumental problems that would be observed with the direct analysis of vegetable oil samples.

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