



Analytical Methods

Combination of supramolecular solvent-based microextraction and ultrasound-assisted extraction for cadmium determination in flaxseed flour by thermospray flame furnace atomic absorption spectrometry

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ABSTRACT

This study describes the development of a new analytical method for cadmium determination in flaxseed flour based on ultrasound-assisted extraction combined with supramolecular preconcentration followed by thermospray flame furnace atomic absorption spectrometry. Cadmium from flaxseed flour was extracted by ultrasound-assisted radiation in acid medium (1.5 mol L⁻¹HNO₃) followed by liquid-liquid microextraction of the acid extract with dodecanoic acid/THF supramolecular solvent using diethyl dithiophosphate as a chelating agent. The limit of detection and the analytical curve range were found to be 0.10 µg L⁻¹ and 0.35 to 20.0 µg L⁻¹, respectively. The cadmium concentration in the flaxseed flours was ranged from 0.11 ± 0.04 to 0.79 ± 0.03 µg g⁻¹. The proposed method is considered simpler, faster, low-cost, and environmentally friendly compared to procedures currently used for the determination of cadmium based on acid digestion and using graphite furnace atomic absorption spectrometry and inductively coupled plasma mass spectrometry.

1. Introduction

The consumption of functional foods is steadily increasing due to the demand of part of the worldwide population for a healthy diet (Venkatakrishnan, Chiu, & Wang, 2019).

Flaxseed (*Linum usitatissimum* L.), which belongs to the family *Linaceae* and also known as linseed, is one of the most important oilseed crops used as functional foods due to its high nutritional value, containing digestible proteins, lignans, and essential fatty acids. Some health benefits include prevention of diseases related to arthritis, menopause, diabetes, cancer, and cardiovascular system (Amit, & Linda, 2010; Oomah, 2001).

Although widely consumed as functional foods, especially as flours, studies have reported the presence of potentially toxic metals, such as cadmium in flaxseed, which contamination source can be attributed to the misuse of pesticides and industrial dumping (Ozkutlu, 2008; Tokaloglu, 2012).

Considering that the European Food Safety Authority (EFSA, 2006)

and the Food and Agriculture Organization and World Health Organization (FAO/WHO, 1995), establish a maximum cadmium limit for cereals of 100.0 µg kg⁻¹, but that there is no specific legislation for maximum cadmium levels in flaxseed, it turns out that analysis and monitoring of cadmium become of paramount importance for flaxseed flour quality control.

The chemical composition of flaxseed may vary widely depending upon climate, soil, types, and cultivars. However, studies reported by the Canadian Grain Commission, the brown Canadian flaxseed contains 41% fat, 20% protein, 28% total dietary fiber, 7.7% moisture, and 3.4% ash (Shim, Gui, Arnison, Wang, & Reaney, 2014).

Owing to the high content of fat and protein, and the very low levels of cadmium in flaxseed, the analytical methodologies usually are based on microwave-assisted digestion with posterior determination by relatively expensive spectroanalytical techniques, such as graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS) (Ozkutlu, 2008; Barreto et al.,

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2018; Zhang et al., 2018). Apart from the high cost of equipment acquisition and maintenance, and the acid consumption for sample digestion, these methodologies are somewhat time-consuming. Therefore, there is still a demand for the development of alternative methods more affordable to most laboratories, with low-cost, faster, easier to perform, and that require smaller quantities of reagents.

Ultrasound-assisted extraction using an appropriated solvent, generally a diluted acid solution, has been successfully applied as an alternative technique in detriment to microwave-assisted digestion for metal extraction from different kind of samples, including soil (Leśniewska, Krymska, Swierad, Wiater, & Godlewska-Zylkiewicz, 2016), edible oil (Kara, Fisher, & Hill, 2015), atmospheric particulate matter (Beal et al., 2018), seafood (Júnior, Krug, Pereira, & Korn, 2006), plant tissue (Krishna & Karunasagar, 2015), alternative oilseed crops (Peronico & Raposo Jr., 2016) and rations for the nutrition of chickens (Barros et al., 2013).

Thermospray Flame Furnace Atomic Absorption Spectrometry (TS-FF-AAS) is considered a powerful technique in terms of detectability when compared with flame atomic absorption spectrometry (FAAS) (Gáspár, & Berndt, 2000; Brancalion, Sabadini, & Arruda, 2007). Besides its easy implementation, this technique requires low sample consumption, which matches with liquid–liquid microextraction procedures that make use of microvolumes of extractor phases.

Among known liquid–liquid microextraction procedures, the supramolecular solvent-based dispersive liquid–liquid microextraction technique (SM-DLLME) (Ballesteros-Gomez, Rubio, & Perez-Bendito, 2009) is one of the most prominent and has been used for a diversity of applications apart from being an environmentally friendly technique (Seidi, Alavi, & Jabbari, 2018; Panhwar et al., 2016). It consists of nanostructured immiscible liquids of amphiphilic compounds (alkanols or alkylcarboxylic acids) that spontaneously self-assemble in water in the presence of THF, which is the continuous phase responsible for the dispersion of the coacervate droplets (Suquilha, Scheel, De Oliveira, & Tarley, 2019; Aydin, Yilmaz, & Soylak, 2015; Karimiyan & Hadjmo-hammadi, 2016; Ruiz, Rubio, & Pérez-Bendito, 2007; Ballesteros-Gómez, Sicilia, & Rubio, 2010). SM-DLLME stands out when compared with cloud point extraction (CPE), due to the spontaneous self-assembly of the amphiphilic compounds in the mixture THF/water, once the water acts as the external stimulus for coacervate formation, while in CPE the use of temperature or the presence of salt is required to form the coacervate droplets.

In view of the aforementioned, the main purpose of this work is to develop a new analytical method for cadmium determination by TS-FF-AAS in flaxseed flour by combining ultrasound-assisted extraction and SM-DLLME. Cadmium was preconcentrated by SM-DLLME, upon ultrasound-assisted extraction, as a metallic complex $[\text{Cd}(\text{DDTP})_2]$. The chelating agent diethyl dithiophosphate (DDTP) presents high affinity towards cadmium and it is able to form a stable complex in acid medium (Ramos, Curtis & Borges, 2013). The analytical method herein proposed has not been reported yet and might be an alternative for those who make use of conventional sample pretreatment procedures based on acid digestion and use relatively expensive atomic/emission spectrometry techniques.

2. Experimental

2.1. Reagents and standards

All reagents utilized were of analytical grade and all working solutions were prepared in ultrapure water (18.2 M Ω cm) from a purification system ELGA® PURELAB Maxima (High Wycombe, Bucks, UK). Diethyl dithiophosphate ammonium salt (DDTP, 95%), nitric acid (HNO_3 , $\geq 65\%$), and HPLC-grade tetrahydrofuran (THF, $\geq 99.9\%$) were purchased from Sigma-Aldrich® (St. Louis, MO, USA). HNO_3 was double distilled with a Berghof® BSB-939-IR sub-boiling system (Eningen, Germany) before utilization. The 1000 mg L^{-1} Cd^{2+} standard solution was

purchased from Quimilab® (Jacareí, SP, Brazil) and the working solutions were properly diluted. For the interference study, solutions of Pb^{2+} , Zn^{2+} , Co^{2+} , Cr^{3+} , Cu^{2+} , Ni^{2+} , Fe^{3+} , Sr^{2+} were prepared from their standard solutions (1000 mg L^{-1}) Quimilab® (Jacareí, SP, Brazil) or their nitrate salts (analytical grade). In order to prevent any possible contamination, all glassware was kept in a 10% (v/v) nitric acid solution for 24 h with posterior cleaning with ultrapure water. Hydrochloric acid (HCl, 37%) from Panreac® (Darmstadt, Germany) and ethanol (EtOH, 99.3%) from Química Moderna® (Barueri, SP, Brazil) were utilized to prepare the carrier solution 1.0 mol L^{-1} HCl:EtOH (1:1, v/v) in the flow system coupled to TS-FF-AAS. Analytical grade hydrogen peroxide (H_2O_2 , 29% v/v) from Synth (Diadema, SP, Brazil) was used in the microwave-assisted digestion of flaxseed flour.

2.2. Apparatus

Cadmium measurements were performed utilizing a flame atomic absorption spectrometer (FAAS) Shimadzu® AA-7000 (Tokyo, Japan) equipped with a cadmium hollow-cathode lamp as the radiation source (wavelength: 228.0 nm; current: 8 mA), a deuterium lamp for background correction, and a flame composition operated with acetylene at flow rate of 2.0 L min^{-1} and air flow rate of 15.0 L min^{-1} . The thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS) system was constituted of an Inconel® 600 tube (Houston, TX, USA) containing a superior central orifice (2.0 mm) to sample injection and six inferior perpendicular orifices (2.0 mm) for flame penetration. The tube (10.0 cm \times 1.0 cm) was placed on stainless support adapted to the FAAS burner, leaving the beam from the lamp pass through its interior. A 0.5 mm i.d \times 2.0 mm e.d ceramic capillary (Al_2O_3 99.7%) (Friatec®, Mannheim, Germany) responsible for generating the spray, was introduced approximately 2.0 mm into the metallic tube. The introduction of the sample in the flow system was performed by using a Gilson® Minipuls Evolution peristaltic pump (Middleton, WI, USA), Tygon® tubes (Courbevoie, France), polyethylene tubes (to propel sample solutions; diameter: 0.8 mm), and a homemade acrylic injector-commutator. The loop volume and carrier flow rate were 43 μL and 0.5 mL min^{-1} , respectively. A vortex oscillator Scilogex® MX-S (Rocky Hill, CT, USA) was utilized to assist the supramolecular solvent-based microextraction procedure and a thermal bath Marconi® MA127 (Piracicaba, Brazil) to separate the phases. An ultrasonic bath Quimis® (Diadema, SP, Brasil) (50 / 60 Hz frequency with 2.4 L capacity) was used for cadmium extraction from flaxseed flours. The acid digestion of samples was carried out by using a Milestone Inc® Ethos Plus microwave oven (Sorisole, Italy).

2.3. Flaxseed flour samples

The samples of commercial flaxseed flour were all purchased from local supermarkets in the city of Londrina (Paraná, Brazil). A total of six samples of different brands and types were purchased (Brown Flaxseed and Golden Flaxseed). The samples were named as letters A, B, C, D, E and F as shown in Fig. S1. It must be pointed out that samples were not water washed, ground and sieved due to high content of fat. Such procedure was adopted aiming at analyzing the samples in the form as they are commercialized.

2.4. Analytical procedure based on ultrasound-assisted extraction combined with supramolecular preconcentration

Initially, the samples were oven-dried at 60.0 °C for 24 h and then 200 mg was weighed into Falcon® tubes of 50.0 mL capacity, followed by adding 20.0 mL of 1.5 mol L^{-1} HNO_3 and then the mixture was kept in an ultrasound bath for 20 min. Upon cadmium leaching from flaxseed flour, the sample was centrifuged and 10.0 mL of supernatant was taken, mixed with 400 μL of DDTP at 41.6% (m/v), and stirred for 0.5 min in vortex for Cd-DDTP complex formation. Afterward, 1.09 mL of

dodecanoic acid at 45.3 mg mL⁻¹ concentration dissolved in THF was added to the solution and, subsequently, subjected to stirring for 1.0 min in vortex. The mixture was then kept in a water bath for 1.0 min at 60.0 °C for microemulsion breaking and then brought to the freezer for rich phase solidification constituted of dodecanoic acid and Cd-DDTP. The poor phase (aqueous phase) was discarded and the rich one adhered to the inner wall of the tube at the upper was dissolved in 250 µL of ethanol:water (1:1 v/v). Then, 47 µL of the sample was introduced into the FIA-TS-FF-AAS using a mixture of 1.0 mol L⁻¹ HCl:ethanol as carrier solution.

2.5. Microwave-assisted digestion of flaxseed flour and optimization of ultrasound assisted-extraction

The efficiency of the cadmium ultrasound-assisted extraction from flaxseed flour was compared with microwave-assisted digestion. For this task, the sample (E), which contained the higher concentration of cadmium was microwave-assisted digested and analyzed by FIA-TS-FF-AAS. The obtained result was then compared, in terms of recovery (%), with ultrasound-assisted extraction during the optimization procedure with direct FIA-TS-FF-AAS determination. It must be emphasized that for samples A, C, D, and F, which contained the lowest cadmium concentration, the comparison was carried out between the proposed method including the ultrasound-assisted extraction, supramolecular

preconcentration and FIA-TS-FF-AAS determination with microwave-assisted digestion and direct FIA-TS-FF-AAS determination.

Before microwave-assisted digestion of flaxseed flours, they were previously oven-dried at 60.0 °C for 24 h, and then 600 mg was weighed into Teflon® flasks and kept overnight with a mixture of 10.0 mL of concentrated HNO₃ and 2.0 mL H₂O₂ (29.0%). The microwave-assisted acid digestion procedure was performed as follows: step 1, ramp 80 °C for 5 min and power of 750 W, step 2, ramp 120 °C for 2 min and power of 750 W, step 3, ramp 190 °C for 10 min and power 1000 W and step 4, ramp 190 °C for 15 min and power 1000 W (Tokaloğlu, 2012). After sample digestion, the excess acid was evaporated on a hot plate to near dryness, the residue was immediately dissolved in ultrapure water and then transferred to a 25.0 mL volumetric flask and analyzed by FIA-TS-FF-AAS.

The optimization of ultrasound-assisted extraction was performed using HNO₃ and HCl as leaching solvents, at different concentrations of 0.50, 1.00 and 1.50 mol L⁻¹. The sample (E) was oven-dried at 60.0 °C for 24 h and then 200 mg was weighed into Falcon® tubes of 50.0 mL capacity, followed by adding 20.0 mL of HNO₃ or HCl solution at different concentrations and left in the ultrasound bath (5 to 40 min). It is noteworthy that the influence of the position of Falcon® tubes in the bath was investigated (Fig. S2). This experiment was performed using a 0.2% (v/v) anionic surfactant solution in 2.0 L of water, a water recirculation at 14.0 mL min⁻¹ by using a peristaltic pump and a numbered

Table 1

Variables, levels, and analytical responses of 2⁶⁻¹ fractional factorial design with a central point for screening the effect of variables on SM-DLLME.

Variables		Levels					
		Low (-1)		0		High(+1)	
Dodecanoic Acid (mg mL ⁻¹)		1.07		2.68		4.3	
THF (µL)		400		700		1000	
DDTP (% m/v)		0.1		1.05		2.0	
NaCl (% m/v)		0		1.0		2.0	
Bath time (min)		1.0		3.0		5.0	
Vortex time (min)		1.0		2.5		5.0	
Experiments	Dodecanoic Acid	DDTP	NaCl	Bath time	Vortex time	THF	Absorbance (Peak Height)
1	-1	-1	-1	-1	-1	-1	0.070
2	1	-1	-1	-1	-1	1	0.329
3	-1	1	-1	-1	-1	1	0.449
4	1	1	-1	-1	-1	-1	0.455
5	-1	-1	1	-1	-1	1	0.046
6	1	-1	1	-1	-1	-1	0.129
7	-1	1	1	-1	-1	-1	0.079
8	1	1	1	-1	-1	1	0.653
9	-1	-1	-1	1	-1	1	0.342
10	1	-1	-1	1	-1	-1	0.071
11	-1	1	-1	1	-1	-1	0.175
12	1	1	-1	1	-1	1	0.608
13	-1	-1	1	1	-1	-1	0.038
14	1	-1	1	1	-1	1	0.184
15	-1	1	1	1	-1	1	0.239
16	1	1	1	1	-1	-1	0.238
17	-1	-1	-1	-1	1	1	0.128
18	1	-1	-1	-1	1	-1	0.281
19	-1	1	-1	-1	1	-1	0.194
20	1	1	-1	-1	1	1	0.623
21	-1	-1	1	-1	1	-1	0.194
22	1	-1	1	-1	1	1	0.135
23	-1	1	1	-1	1	1	0.166
24	1	1	1	-1	1	-1	0.269
25	-1	-1	-1	1	1	-1	0.177
26	1	-1	-1	1	1	1	0.061
27	-1	1	-1	1	1	1	0.403
28	1	1	-1	1	1	-1	0.448
29	-1	-1	1	1	1	1	0.155
30	1	-1	1	1	1	-1	0.104
31	-1	1	1	1	1	-1	0.139
32	1	1	1	1	1	1	0.533
33	0	0	0	0	0	0	0.734
34	0	0	0	0	0	0	0.68
35	0	0	0	0	0	0	0.619

home holder containing thirteen positions for 50.0 mL tubes.

2.6. Multivariate optimization of SM-DLLME procedure

The influence of variables that play an important role on the SM-DLLME procedure, including the concentration of the amphiphilic compound (dodecanoic acid) (1.07 to 4.3 mg mL^{-1}), dispersing agent volume (THF) (400 to $1000 \text{ }\mu\text{L}$), concentration of the chelating agent DDTP (0.1 to 2.0% w/v), NaCl concentration (0 to 2.0% w/v), water bath time (1 to 5 min) and vortex time (1 to 5 min) were evaluated using a two-level 2^{6-1} factorial design containing a central point carried out in triplicate to estimate the experimental error (Table 1). Graphically the influence of the variables was analyzed through a Pareto chart, at a 95% confidence interval (Teófilo, & Ferreira, 2006).

After the application of the 2^{6-1} fractional factorial design, the final optimization was carried out by using a two-variable Doehlert matrix with the most important variables, DDTP and THF volume (Ferreira, dos Santos, Quintella, Neto, & Bosque-Sendra, 2004). A response surface was then used to obtain the optimum condition, whose validation of the quadratic model was attested by analysis of variance (ANOVA) at a 95% confidence interval. The central point of Doehlert matrix was performed in sextuplicate, the sample volume was set as 10.0 mL and the cadmium concentration was set as $10.0 \text{ }\mu\text{g L}^{-1}$. Statistical data were processed in a StatSoft Statistica 7.0 software package.

2.7. Assessment of analytical performance of the method

Under optimized conditions and using a 10.0 mL preconcentration volume, the analytical performance of the method was analyzed in terms of linearity of the analytical curve, preconcentration factor, consumption index, preconcentration efficiency, limit of detection (LOD) and quantification (LOQ), intra and interday precision and accuracy. All analytical parameters were determined according to the International Union of Pure and Applied Chemistry (IUPAC) (Currie, 1995) and/or according to the literature (Souza & Tarley, 2009; Gorji, Biparva, Bahram, & Nematzadeh, 2019; Kashanaki, Ebrahimzadeh, & Moradi, 2018). The linear analytical curve was built in the range of 0.35 – $20.0 \text{ }\mu\text{g L}^{-1}$ in triplicate for each concentration. Preconcentration factor (PF) is defined as the ratio between the linear regression slope obtained by SM-DLLME method and the linear regression slope of the direct determination by FIA-TS-FF-AAS or FAAS. The consumption index (CI) was calculated by the ratio between the sample volume (10.0 mL) and the preconcentration factor (PF). The preconcentration efficiency (EP) is defined as the ratio between preconcentration factor (PF) and sample volume (10.0 mL). The LOD and LOQ were determined as $3\text{SD}/s$ and $10\text{SD}/s$, respectively, where SD is the standard deviation of ten blank measurements, and s is the slope of the analytical curve. The intraday precision was assessed in terms of repeatability for 10 determinations of cadmium solutions at 0.50 and $16.00 \text{ }\mu\text{g L}^{-1}$ concentrations. For interday precision, the 10 determinations were performed on two consecutive days.

For assessing the accuracy of the method, the content of cadmium in flaxseed flours was compared with that obtained by using microwave-assisted digestion and direct TS-FF-AAS determination.

3. Results and discussion

3.1. Ultrasound-assisted extraction of cadmium from flaxseed flour

Ultrasonic bath is the most available and cheapest source of ultrasonic irradiation (Capelo, 2005). However, the efficiency of ultrasound-assisted extraction depends on cavitation efficiency, which in turn depends on the leaching solvent, the position of the sample within the bath, and the extraction time (Capelo, 2005; Shirsath, Sonawane, & Gogate, 2012).

Therefore, the influence of HCl and HNO_3 solutions at different concentrations (0.50 – 1.50 mol L^{-1}) as leaching solvents, as well as the

sample positions into the bath on the ultrasound-assisted extraction efficiency were evaluated.

As depicted in Fig. 1A and 1B, the lowest concentration of the leaching solvents is not appropriated to quantitative extract cadmium from the flaxseed flour. On the other hand, the use of both HCl and HNO_3 at 1.5 mol L^{-1} concentration is appropriated to achieve good extraction with recovery percentages higher than 90% for all investigated bath positions. The good performance of HCl and HNO_3 for ultrasound-assisted extraction is somewhat expected once the first one has complexing properties, while the last one is an oxidant acid. Such features are very important to assist the metal release from the food matrix. Although both acids might be used in the extraction procedure, HNO_3 at 1.5 mol L^{-1} concentration was chosen for further experiments, due to the high solubility of metallic nitrates and for not being one complexing acid, which in turn could influence the SM-DLLME procedure. One should note that under this condition, all thirteen ultrasound bath positions might be simultaneously used with equal efficiency, which demonstrates good robustness of the extraction method mainly if one considers that samples were not ground and sieved. Additionally, the use of all bath positions improves the analytical frequency of the method.

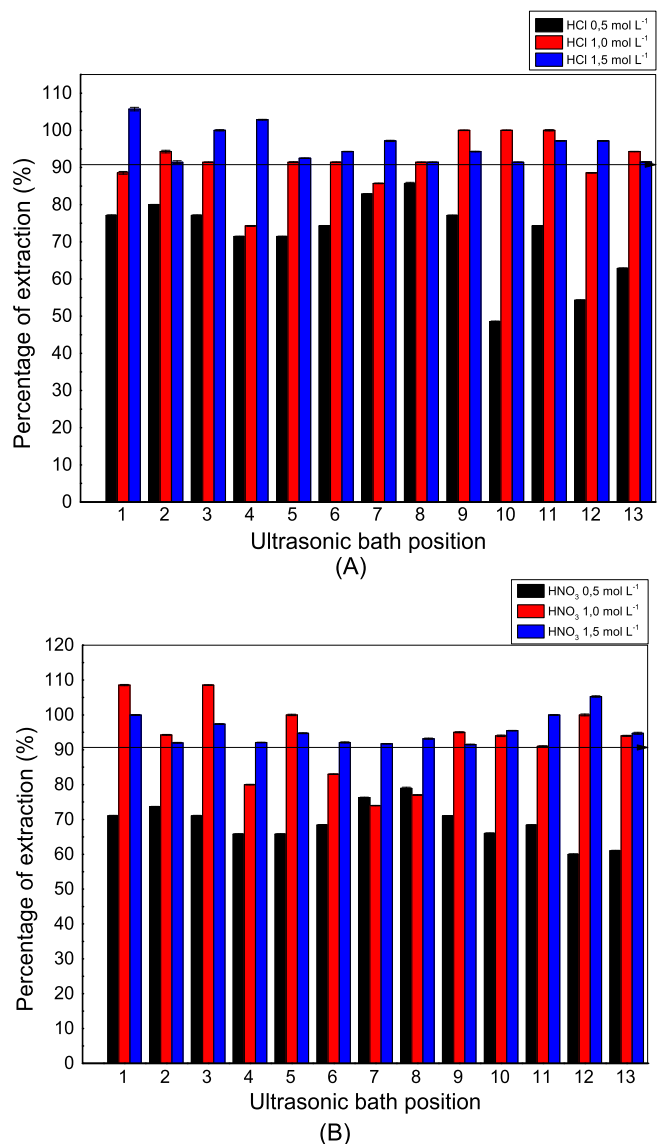


Fig. 1. Influence of HCl concentrations (A) and HNO_3 concentrations (B) and ultrasound bath position on the extraction of cadmium from flaxseed flour.

The ultrasound-assisted extraction time dependence was investigated in the range of 5–40 min (Fig. S3). The obtained results indicated that the extraction time of 20 min provides quantitative extraction of cadmium. Therefore, an ultrasonication extraction time of 20 min was selected as being optimal to leach cadmium from the flaxseed flour to the extraction phase.

3.2. Effect of HNO_3 concentration on SM-DLLME

The pH of the sample solution plays an important role in the metal-chelate formation and transfer to the extraction phase (Aydin et al., 2015; Coelho, & Arruda, 2005). However, it is worth emphasizing that, based on the sequential combination of ultrasound-assisted extraction and SM-DLLME in a single analytical method, it is crucial to obtain an extraction medium compatible with the SM-DLLME procedure. As shown in Fig. S4, the highest absorbance of cadmium when subjected to the SM-DLLME procedure was obtained by using the lowest acid concentration ($0.5 \text{ mol L}^{-1} \text{ HNO}_3$), since the use of a strong acid medium makes the metal-chelate formation less stable. Although the use of $0.5 \text{ mol L}^{-1} \text{ HNO}_3$ offers an optimum acid medium for SM-DLLME, it is not appropriated for leaching cadmium from flaxseed flour as already shown in Fig. 1B. Therefore, as a compromise between sensibility by SM-DLLME procedure and quantitative leaching of cadmium from the flaxseed flour, HNO_3 at 1.5 mol L^{-1} concentration was also chosen for the SM-DLLME procedure.

3.3. SM-DLLME optimization

In order to determine the best preconcentration conditions of the SM-DLLME method, the optimization was carried in two steps by using a two-level 2^{6-1} fractional factorial design with a central point for screening the main effect of variables followed by a Doehlert multivariate technique.

Table 1 shows the experimental matrix containing the variables, their levels, and the analytical responses (Absorbance) of cadmium. Fig. 2 depicts the Pareto chart, where the curvature was found to be

statistically significant with a negative standardized estimated effect (-8.90) at a 95% confidence interval, thereby indicating that the best analytical response is near the central point of the experiments.

With exception of the water bath and vortex time, all the other variables exerted statistically significant influence ($p < 0.05$) on the SM-DLLME. The concentration of chelating agent DDTP, dodecanoic acid, and the volume of THF were the most important variables with a positive standardized estimated effect of 7.23, 4.76, and 4.46, respectively. Increasing DDTP concentration increases the Cd-DDTP formation and, as a consequence, the efficiency of extraction. On the other hand, using higher DDTP concentration ($2.0\% \text{ m/v}$), competition between DDTP and Cd-DDTP into the self-assembled dodecanoic acid might occur.

Regarding the concentration of dodecanoic acid, as its concentration was increased within the experimental domain (1.07 to 4.3 mg mL^{-1}), higher efficiency extraction of Cd-DDTP was observed even with the dilution of the rich phase. Therefore, the concentration of 4.3 mg mL^{-1} was chosen as the optimum condition once higher concentrations were not soluble in THF.

The positive standardized estimated effect of THF volume allows us to infer two conditions: THF plays a double role on supramolecular extraction because it acts as disperser solvent and promotes self-assembly of dodecanoic acid. In this way, increasing the THF volume increases the dispersion of supramolecular solvent with consequent improvements in the extraction efficiency. However, a higher volume of THF results in a decrease of the supramolecular solvent concentration, thereby impairing the extraction.

When NaCl was added to the solution, the extraction efficiency of Cd-DDTP into the supramolecular solvent was decreased, as observed by a negative standardized estimated effect of -3.39 . The reason for this result can be attributed to decrease of diffusion kinetics of Cd-DDTP into the organic phase, as a result of high ionic strength, as well as due to disruption of original charge distribution of amphiphilic molecules, which in turns hinders the coacervate formation (Beal et al., 2018; Ruiz et al., 2007). Therefore, further studies were carried out in the absence of NaCl.

After screening the variables from the two-level 2^{6-1} fractional

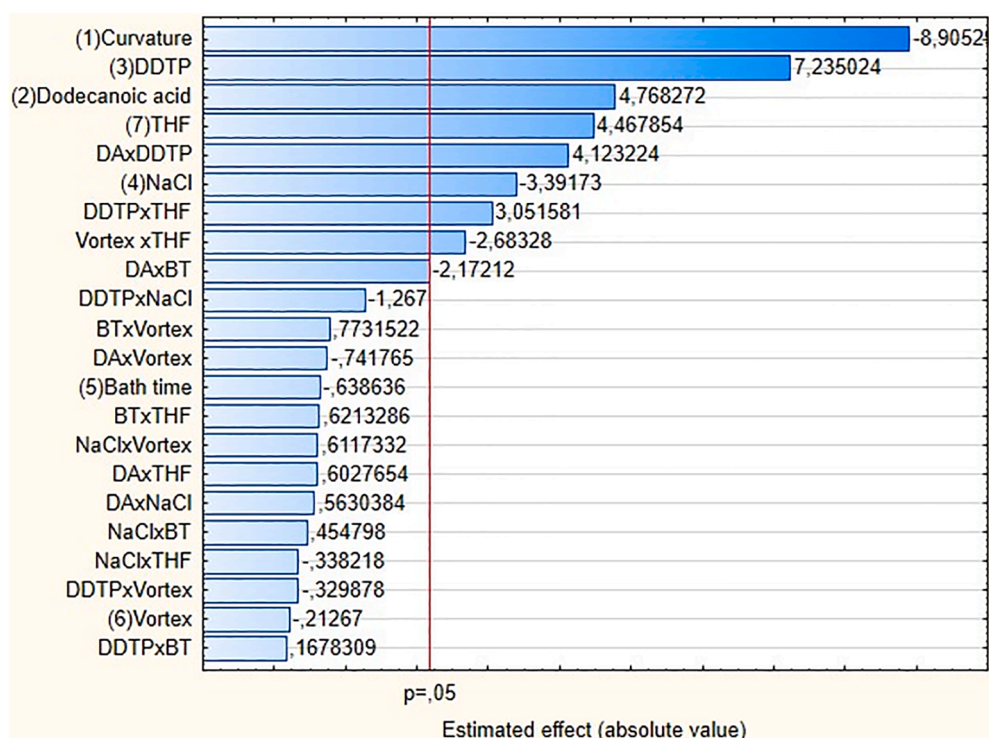


Fig. 2. Pareto chart obtained from 2^{6-1} fractional factorial design for screening the effects of variables on the SM-DLLME procedure.

factorial design, the final optimization of DDTP concentration and THF volume was performed by Doehlert matrix (Table S1). The assays were carried out by setting the water bath and vortex time at 1 min, in the absence of NaCl, and using dodecanoic acid at 4.3 mg mL⁻¹ concentration.

The quadratic equation model (equation 1) obtained from the Doehlert matrix was validated by analysis of variance (ANOVA) (Table S2). The model showed no lack of fit due to the tabulated value $F_{1,5}$ (6.61) is higher than the calculated values of F_{cal} (0.366), a 95% confidence level.

$$y(Abs) = -0.07370 + 0.2951(DDTP) - 0.0593(DDTP)^2 + 0.9163(THF) - 0.3434(THF)^2 - 0.1104(DDTP \times THF) \quad (1)$$

The surface response obtained using the validated quadratic model is depicted in Fig. 3, where the maximum analytical signal for cadmium was obtained using 1.09 mL of THF and 1.46% (m/v) of DDTP.

3.4. Interference study

Under optimized conditions, the effect of ions commonly present in flaxseed flour samples capable to form metallic complexes with DDTP was evaluated on the cadmium preconcentration (Tokaloglu, 2012). A solution containing coexisting ions, Cr³⁺ (5.52 µg L⁻¹), Fe³⁺ (313.00 µg L⁻¹), Co²⁺ (1.44 µg L⁻¹), Ni²⁺ (14.56 µg L⁻¹), Cu²⁺ (92.80 µg L⁻¹), Zn²⁺ (532.80 µg L⁻¹), Sr³⁺ (114.40 µg L⁻¹) and Pb²⁺ (0.32 µg L⁻¹) and 10.0 µg L⁻¹ of cadmium was subjected to SM-DLLME procedure, which was carried out in triplicate. The tolerance limit was defined as the concentration of coexisting ions that cause a change in the cadmium signal alone of ± 10.0%. A relative error of 5% was obtained, which indicates that there were no interferences from ions commonly present in flaxseed flour. It is important to point out that some alkaline and alkaline-earth metals, such as Mg²⁺, Na⁺, K⁺, Ba²⁺, and Ca²⁺ were not investigated because they do not form metallic complexes with DDTP (Ramos et al., 2013).

3.5. Analytical performance of SM-DLLME method

The linearity of the analytical curve in the range of 0.35–20.0 µg L⁻¹ was evaluated by analysis of variance (ANOVA) at the 95% confidence level. The summary of ANOVA of the regression model is presented in Table S3. As shown, the MS_R/MS_{res} ratio defined by F_{cal} of 1023.27 was much higher than F_{tab} (4.38), revealing that the linear model seems to be the most suitable to describe the linear correlation between the absorbance and the concentration of Cd²⁺ in the range of 0.35–20.0 µg L⁻¹.

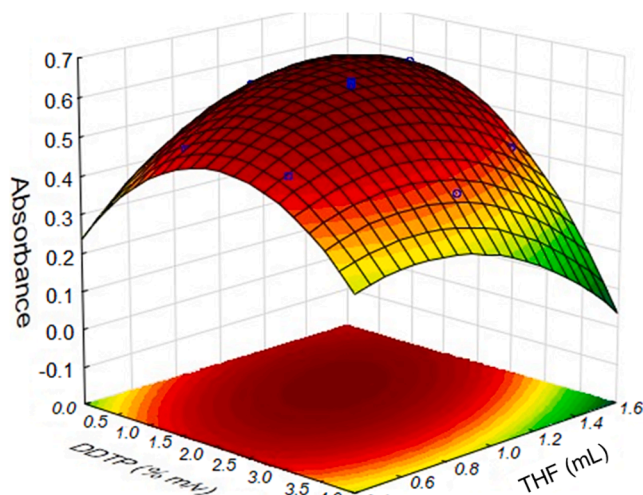


Fig. 3. Response surface obtained from Doehlert matrix.

Additionally, the MS_{lack}/MS_{pe} ratio (F_{cal} of 1.30) was lower than the F_{tab} value of 2.96, indicating the absence of lack of fit of the linear model. The LOD and LOQ values obtained by the proposed method were found to be 0.10 and 0.35 µg L⁻¹, respectively. Considering the use of 200 mg of flaxseed flour in the development of the method, the obtained LOD and LOQ in µg Kg⁻¹ were found to be 5.0 and 17.5, respectively.

The preconcentration factor (PF), the preconcentration efficiency (PE), and the consumption index (CI) were found to be 8.72 times, 0.87 mL⁻¹, and 1.15 mL, respectively. When the sensitivity of the method was compared to that obtained by direct aspiration of Cd²⁺ ions into FAAS nebulizer, a remarkable sensitivity improvement of 146 times was obtained.

The intraday precision assessed as the percentage of relative standard deviation (RSD, %) of 7.34 and 4.61% for the respective concentrations of 0.50 and 16.0 µg L⁻¹ of cadmium was obtained. The obtained values for interday precision ranged from 3.12 to 4.78%.

The analytical parameters of the method have been compared to previously published methods for cadmium determination by TS-FF-AAS involving different preconcentration approaches (Table 2). As observed, the developed method shows LOD comparable to other reported methods, mainly if one considers the low sample consumption of 10.0 mL. Additionally, in comparison to the solid-phase extraction methods, which require previous microwave-assisted digestion of samples, the sample preparation using ultrasound-assisted extraction is faster and low-cost. The SM-DLLME preconcentration procedure is also achieved very quickly (1 min of vortex time + 1 min of water bath time), which is lower than the extraction time usually required in CPE methods. Apart from these advantages, it must be emphasized that the ultrasound-assisted extraction combined with SM-DLLME is carried out by using an ultrasound bath, water bath, and vortex oscillator, which are very inexpensive and common instruments in most laboratories.

3.6. Analysis of brown and gold flaxseed flour

The analytical accuracy and applicability of the proposed method were evaluated by analyzing commercial flaxseed flour under optimized conditions. Table 3 shows the obtained results which are in close agreement with microwave-assisted digestion by applying the paired Student's *t*-test with a 95% confidence interval. These results reveal the accuracy of the ultrasound-assisted extraction of cadmium from flaxseed samples and its reliable application in high-fat foods.

It is important to stress out, as already mentioned, that there is no specific legislation for maximum cadmium levels in flaxseed; however, taking into account the maximum level established of cadmium in cereals by the European Food Safety Authority (EFSA, 2006) and the Food and Agriculture Organization and World Health (FAO/WHO, 1995) of 100.0 µg kg⁻¹, the cadmium contents in the flaxseed flour herein determined were found to be much higher. Therefore, simple, reliable, and low-cost quality control protocols must be increasingly studied and implemented to guarantee the quality of foods and the security of the population. Similar results have been obtained for flaxseed cultivated in Turkey (120.0 µg kg⁻¹) (Ozkutlu, 2008), in which cadmium content was determined by ICP-MS after microwave-assisted digestion.

4. Conclusion

The use of ultrasound-assisted extraction combined with SM-DLLME based on the formation of reverse dodecanoic acid micelles dispersed in a continuous phase composed of tetrahydrofuran (THF)/water is an efficient and reliable method for cadmium quantification in commercial flaxseed flour samples using FIA-TS-FF-AAS. The method stands out owing to the low sample consumption, simplicity, use of inexpensive instruments, and being environmentally friendly, thereby being an alternative to the conventionally used sample pretreatment procedures based on acid digestion and those which use relatively expensive atomic spectrometry/emission techniques. As a limitation of ultrasound-

Table 2

Comparison of analytical parameters of the proposed method with other preconcentration methods using TS-FF-AAS as analytical technique.

Method	Reagent	LOD ($\mu\text{g L}^{-1}$)	Sample Volume (mL)	Linear range ($\mu\text{g L}^{-1}$)	Samples	References
Solid-phase extraction on MWCNT-polyvinylpyridine	–	0.036	27.0	0.12–6.0	Waters, cigarettes, chocolate, medicinal herb	Tarley, Diniz, Suquila, & Segatelli, 2017
HF-SLME	APDC	0.009	100.0	0.04–2.0	Water samples, hair and tea	Zeng, Hu & Luo, 2012
Cloud point extraction	Br-PADAP	–	10.0	0.08–25.0	Tobacco	Andrade, Nascentes & Costa, 2009
On-line precipitation–dissolution in knotted reactor	Ammonia	0.13	4.0	0.13–2.0	Rice and water samples	Wen, Wu, Xu, Wang, & Hou, 2009
Dispersive solid-phase extraction with TiO_2	Dithizone	0.04	10.0	0.05–15.0	Water samples	Yang, Jiang, Hu, & Wen, 2020
SM-DLLME	DDTP	0.10	10.0	0.35–20.0	Flaxseed flour	This Work

LOD: limit of detection; APDC: Ammonium pyrrolidine dithiocarbamate; DDTP: ammonium diethyl dithiophosphate; MWCNT: Multiwall Carbon Nanotube; HF-SLME: Hollow fiber supported liquid membrane extraction; SM-DLLME: Supramolecular solvent; Br-PADAP: 2-(bromo-2-pyridylazo)-5-diethyl-amino-phenol.

Table 3

Determination of cadmium in commercial flaxseed samples by the proposed method (ultrasound-assisted extraction combined with SM-DLLME) and comparison with microwave-assisted digestion and TS-FF-AAS determination.

Samples	Concentration of Cd^{2+} ($\mu\text{g g}^{-1}$)		
	Proposed method ^a	Microwave-assisted digestion	$T_{\text{calculated}}$
Sample A	0.12 ± 0.03	0.11 ± 0.04	0.35
Sample B	<LD	<LD	–
Sample C	0.21 ± 0.01	0.20 ± 0.01	1.23
Sample D	0.28 ± 0.02	0.26 ± 0.03	0.98
Sample E	0.76 ± 0.02	0.79 ± 0.03	1.47
Sample F	0.29 ± 0.02	0.28 ± 0.02	0.61

<LD: Below of limit of detection; ^aResults expressed as mean \pm standard deviation based on triplicate (n = 3).

assisted extraction combined with SM-DLLME, it can be cited the low robustness for a wide variety of samples once the efficiency of the method depends upon the nature of the sample and metallic ion.

For final remarks, since the presence of cadmium in flaxseed flour samples was observed in rather high levels, considering the maximum level established for cadmium in cereals, as well as the increased consumption of this type of functional food, it becomes increasingly important to assess the presence of toxic metals to assure the quality control of foods and benefits to human health.

CRediT authorship contribution statement

Leonardo Francisco Rafael Lemes: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - original draft. **César Ricardo Teixeira Tarley:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2021.129695>.

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