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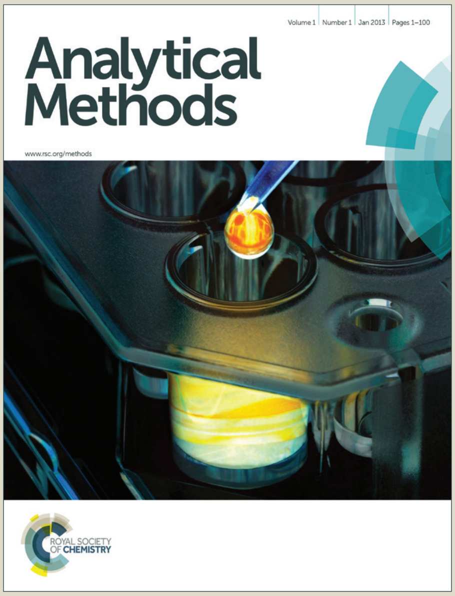

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Ultrasound-assisted digestion of biodiesel samples for metal determinations by stripping voltammetry

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Abstract

The ultrasound-assisted digestion of biodiesel samples is demonstrated for the first time. Efficiency of the proposed sample preparation method was evaluated for trace metal determination by stripping voltammetric techniques, which are more susceptible to residual carbon content (RCC) in digestates. Biodiesel samples (50–75 mg) were treated in glass tubes, with a mixture of 3.0 mL of concentrated HCl and 4.5 mL of H_2O_2, placed inside an ultrasonic bath (40 kHz frequency and 120 W) exactly over the region of higher ultrasound intensity and sonicated for 40 min. These parameters were optimized using factorial design experiments for the analysis of spiked samples. Recovery values between 94 to 108 % for zinc, cadmium, lead, copper and mercury were verified under optimized conditions. RCC values after US-assisted digestions were in the range of 3.05-3.75% using covered glass tubes, which are acceptable values for the accurate voltammetric determination of metals. These results indicate that the proposed sample preparation method presents comparable performance to traditional or microwave-assisted acid digestions and can associated with on-site electroanalysis using screen-printed electrodes.

Keywords: Factorial design experiment; Ultrasound; Residual carbon; Screen-printed electrode.

Introduction

Ultrasound-assisted (US-assisted) digestions or extractions have been ~~extensive~~ extensively investigated as an alternative procedure of sample preparation aiming metal determination in a large range of solid and liquid samples by spectrometric and electroanalytical techniques [1]. The main advantages of applying the ultrasound energy for sample preparation include the simple instrumentation (ultrasonic probes or baths), high-speed treatments, and safer conditions for the analyst (operating at atmospheric pressure and room-temperature). Based on these features, US-assisted treatments have been proposed to replace the traditional wet digestion or dry ashing procedures and even microwave-assisted digestions for metal determinations [2-4].

The metal content is an essential parameter for the quality control in fuels. The presence of trace metals is associated with the formation of gums and sediments inside vehicle engines and storage tanks. Although there are still no rules in legislations for several metallic species present in biodiesel, such as Cd, Cu, Fe, Ni, Pb, Zn, these metals can accelerate oxidation processes of biodiesel [5-7]. The tendency of oxidative degradation of biodiesel is measured by the Rancimat method, which consists in the measurement of the oxidation process induced by the heating of a biodiesel sample at 110°C in the presence of continuous airflow. Volatile oxidation products are trapped in another flask containing deionised water, causing an increase in conductivity that determined the end of the measurement. According to EN14112, the minimum induction time of 6 h is required for a biodiesel sample within the norm. Therefore, the determination of metals in biodiesel would give a fast evidence of degradation processes in biodiesels. Metals in biodiesel can occur due to corrosion of metallic alloys commonly found in engines and storage tanks and can also be introduced from the source used for biodiesel production [8,9].

Analytical methods reported for metal determination in biodiesel typically employ atomic absorption spectrometry (AAS) and stripping analysis after adequate sample preparation [9,10]. Microwave-assisted digestion and wet digestion procedures have been described for the determination of metals and metalloids in biodiesel. Dry ashing was used for Sn determination in biodiesel by stripping analysis [11] while microwave-assisted digestion procedures were reported for the determination of Ca [12] and Cd, Cu, Pb and Zn [13] in biodiesel by stripping analysis. Electrothermal atomic absorption spectrometry (ET-AAS) determination of Cd and Hg was accomplished after microwave digestion of biodiesel [14] as well as ET-AAS determination of Cu, Pb, Ni and Cd [15]. Similarly, inductively-coupled plasma optical emission spectrometry (ICP-OES) determination of Ca, P, Mg, K, and Na was performed after microwave digestion [16]. Therefore, metal determination in biodiesel by either stripping analysis or spectrometric methods generally requires a sample preparation step and microwave-assisted digestions have been constantly reported. However, a microwave oven is not always available in analytical laboratories and the typical digestion procedures take at least 30 min plus 20 min for cooling down the temperature of flasks inside the oven.

In this work, a reasonably fast US-assisted treatment using an ultrasonic bath is proposed for metal determination in biodiesel samples. Stripping analysis was selected for trace determination of Zn, Cd, Pb, Cu and Hg, once this technique are commonly affected by residual organic matter. Thus, the proposed method could also be extended for metal determinations by spectrometric techniques.

Experimental

Reagents, stock solutions and bioethanol samples

High-purity deionized water (R ≥ 18 MΩ cm) obtained from a Milli Q water purification system (Millipore, Bedford, MA, USA) was used for preparing all aqueous solutions. Concentrated hydrochloric (37% w/v), acetic (99% w/v) and nitric (65% w/v) acids were obtained from Vetec (Rio de Janeiro, Brazil). Sodium acetate (98.0% w/w) and ethanol (99.5% v/v) were acquired from Synth (Diadema, Brazil). Cadmium, copper, lead, zinc and mercury stock solutions (1000 mg L^{-1}) were purchased from Quimlab (Jacareí, Brazil). Hydrogen peroxide (30% w/v) was obtained from Audaz (São Paulo, Brazil).

Hydroethanolic standard solutions (95.0% v/v ethanol and 5.0% v/v water) of metals were prepared for fortification of biodiesel samples used for optimization of the digestion process. Aqueous solutions of metals were prepared separately for standard addition experiments for obtaining the analytical calibration curves. Working standard solutions were prepared just before use by appropriate dilution of the stock solution acidified with 1% (v/v) HNO_3. All reagents were used without further purification (analytical grade). Aqueous solution of 0.5 mol L^{-1} sodium acetate was added to the acid digestates in order to generate acetate buffer solution as electrolyte for the electrochemical cell measurements and avoid hydrogen evolution due to the very negative potentials applied.

Samples of methyl biodiesel (produced from soybean oil) used in this work were provided by Caramuru industry, located at São Simão, GO, Brazil. Biodiesels produced from different oil sources such as tallow, colza, sunflower, corn, residual cooking oil, cottonseed were synthetized in the laboratory by methyl transesterification using alkaline catalyst [17,18]. Vegetable oils were obtained at local markets and recycled cooking oil donated by local restaurants. The biodiesel samples were stored in amber flask at room temperature (20±1°C) encased in aluminum foil. All materials of glass and plastic used for the preparation, storage and digested of the samples were decontaminated by immersion in nitric acid 10% (v/v) for 24 h. Digested samples were stored in polyethylene vessels left at room temperature.

Instrumentation, electrochemical cell and electrodes

For sample preparation, a Unique UltraCleaner 1640A ultrasonic bath with a capacity of 3.8 L, 40 kHz frequency and 120 W of potency (São Paulo, Brazil) and glass flasks ~~(Pyrex No.9825)~~ of 50 mL of internal volume were used.

For electrochemical measurements was employed a µ-Autolab type III potentiostat (EcoChemie, Utrecht, Netherlands) interfaced to a microcomputer with GPES software, version 4.9.

A homemade cell (with internal volume of 10 mL) made of glass was used for the electrochemical measurements. The digested samples of biodiesel were analyzed using a platinum wire as auxiliary electrode, a miniaturized $Ag/AgCl_{(sat)}$ reference electrode [19], and a 3.0 mm diameter glassy-carbon electrode (GCE). Mercury film was electrodeposited at the GCE surface by applying a potential of -800 mV for 300 s in a solution containing 100.0 mg L^{-1} mercury (II) and 0.1 mol L^{-1} HCl [20]. Before the electrodeposition of mercury film inside the electrochemical cell, the GCE surface was previously cleaned by mechanical polishing with alumina (0.1 µm particle) and then washed with deionized water.

Disposable gold screen-printed electrodes (Au-SPEs) were used for mercury determination in the digestates. Each SPE strip (C220BT, Dropsens, Oviedo, Spain) contains the three electrodes at the same ceramic substrate, at which a gold ink is printed as working and counter electrodes and a silver ink as the pseudo-reference electrode. These SPEs were adapted to a batch-injection (BIA) cell in a configuration of a fully portable electrochemical system [21]. The BIA system is analogous to flow-injection analysis (FIA), in which pump and valves of the FIA system are replaced by micropipette (it can be an electronic micropipette) that injects a sample plug (few microliters) directly onto the working electrode immersed in supporting electrolyte [22,23].

Electrochemical measurements

The analysis of biodiesel samples spiked with zinc was performed by stripping chronopotentiometry. Deposition potential and stripping time were -1.4 V and 30 s, respectively, scanning from -1.4 to -0.5 V. After obtaining the optimized procedure for the US-assisted digestion of biodiesel samples, square-wave anodic stripping voltammetry (SWASV) was used for the determination of zinc, cadmium, lead, and copper in the digested samples. The SWASV parameters are described in Table 1. The obtained detection limits for Cd, Cu, Pb, and Zn were 4.5, 2.2, 6.7, and 7.5 µg Kg $^{-1}$, respectively. Mercury determination in the digestates was performed by SWASV (conditions in Table 1) using the Au-SPE in the BIA system. The optimized BIA parameters were 200 µL as sample injection volume and 53 µL s^{-1} as dispensing rate, which occurs during the deposition step. The obtained detection limit for Hg under these conditions was 4.0 µg Kg $^{-1}$.

INSERT TABLE 1

All electrochemical measurements were carried out in the presence of dissolved oxygen and at room temperature. The standard addition method was used for all analyses.

Optimization of US-assisted digestion procedure of biodiesel samples

The mixture of acids/oxidants and biodiesel samples inside glass flasks were placed in the central region of the bath that corresponds to the region where the piezoelectric crystals, responsible for the bath sonication process, are located [24].

Concentrated 37% (w/v) HCl, 30% (w/v) H_2O_2, or a mixture of both reagents was evaluated to digest biodiesels within ultrasonic baths. Other variables that influence on the efficiency of biodiesel digestion samples were evaluated by 2^2 factorial design experiments [25]. The variable volumes of HCl and H_2O_2 in the ranges of 2.0–4.0 mL of both HCl and H_2O_2 were first optimized using a 2^2 factorial design experiment, which consisted of 11 experiments. Next, the temperature of the bath and sonication time were optimized in the ranges of 20–40 °C and 20–40 min using a 2^2 factorial design experiment, which also consisted of 11 experiments. A thermometer was placed in the ultrasonic bath to control its temperature (variation of ±1°C). The conditions of both factorial design experiments are described in Table 2. The biodiesel mass was optimized following univariate conditions using the following mass values, 50, 100, 150, 200, and 250 mg. The effect of tightly closing the glass tubes and just covering the tubes under sonication was evaluated. After sonication, the digestates were left to stand for about 20 minutes at room temperature to eliminate excess of bubble generated during the digestion. Aliquots of the digest were then taken for perform the analysis in aqueous medium.

INSERT TABLE 2

Analysis of residual carbon content

The amount of 50 mg of pure methyl biodiesels (tallow, colza, sunflower, corn, waste cooking oil, and cottonseed) was US-assisted digested using 3.0 mL of concentrated HCl and 4.5 mL of H_2O_2 30% (v/v) under optimized conditions inside closed tightly glass tubes and inside covered glass tubes. The digests were diluted to 20.0 mL with deionized water. Next, the samples were pre-filtered with Millex LCR filter to remove particulate material and then placed in the sample compartment. The measuring of the residual carbon content was performed in a TOC analyser (Shimadzu TOC-VCPH/CPN) equipped with an ASI-V auto sampler. The samples were introduced one by one automatically into the combustion tube apparatus, which was heated to a temperature of 680 °C. Then, the carrier gas (O_2) carried at a rate of 150 mL min^{-1}, resulting in the combustion of the samples. The combustion products were carried to an electronic dehumidifier, where they were cooled and dried. The carrier gas then carried the combustion products through a tube where chlorine and other halogens were removed. Finally, the transported gas carried the combustion products of the sample cell ~~for~~ to a non-dispersive infrared, where CO_2 was detected.

Results and Discussion

Optimization of the ultrasound-assisted digestion

Factorial design experiments were planned to evaluate some variables of the US-assisted treatment of biodiesel samples. A previous work reported the use of a 1:1 (v/v) mixture of concentrated HCl 37% (w/v) and 30% (w/v) H_2O_2 for the US-assisted extraction of metals from lubricating oils using an ultrasonic bath and mapping the regions over the bath of higher ultrasonic intensity [24]. Other authors also proposed the use of an ultrasonic bath for metal extraction from lubrication oils using the same acid/oxidant extractor and included the evaluation of the effect of temperature [26]. Based on these data from the literature, the individual effects of concentrated HCl 37% (w/v) and 30% (w/v) H_2O_2, temperature of the ultrasonic bath and sonication time were the selected variables to be evaluated for the US-assisted treatment of biodiesel samples using 2^2 factorial design experiments. The optimization of these variables were based on the stripping chronopotentiometric determination of zinc in the digestates obtained after biodiesel treatment. The selection of two 2^2 factorial design experiments to evaluate four variables of the US-assisted digestion was planned in order to know the interaction of a pair of variables on the efficiency of the procedure.

The first studied variables using a 2^2 factorial design experiment were the volumes of concentrated HCl 37% (w/v) and 30% (w/v) H_2O_2 and their mixture in accordance with the minimum and maximum levels described in Table 2, keeping constant the temperature of the bath (20 ºC) and sonication time of 30 min for a sample mass of 50 mg. According to the Pareto chart (not shown) and considering a 95% confidence level, both variables of the 2^2 factorial design showed to be statistically significant for the extraction of zinc. The contour lines of the response surface are presented in Figure 1A. This chart shows that the region of higher percentage in recovery of zinc ions (optimum region) ranges from -0.5 to 1.0 of HCl and from 0.5 to 1.5 of H_2O_2, approximately. Inside this region, it was selected the points 0 and 1.5 for HCl and H_2O_2, which corresponded to 3.0 and 4.5 mL, respectively, because small standard deviation values were obtained under these conditions. It is noteworthy to mention that US-assisted treatment of the biodiesel sample leaded to the complete digestion as no residual organic content could be visually observed, oppositely to previous works that reported the metal extraction process from lubricating oils as the sample matrix was not fully digested [24,26]. Moreover, the individual use of HCl or

H_2O_2 did not lead to the digestion of biodiesel samples, as residual organic matrix was observed after the US-assisted treatment. The same can be affirmed if sonication was not applied to biodiesel samples in the presence of the mixture of HCl and H_2O_2. The investigation of residual organic content after US-assisted digestions using the mixture of HCl and H_2O_2 was further presented.

INSERT FIGURE 1

The second studied variables using a 2^2 factorial design experiment were the sonication time and temperature of the bath in accordance with the minimum and maximum levels described in Table 2. Figure 1B presents contour lines of the response surface obtained to evaluate the two variables based on recovery of Zn. This chart shows that there was a negative interaction between sonication time and temperature, which indicated higher recovery values for high sonication time at low temperatures and vice versa. The optimal condition was observed at low temperature (level -1) and higher sonication time (level +1), which corresponded to 40 min and 20 ºC, respectively. Therefore, these conditions were maintained in further experiments.

Mass sample was evaluated in univariate mode under optimized conditions of extractor, sonication time and temperature of the bath. This study revealed that higher amount of biodiesel sample was not successfully digested because residual organic matrix was observed after the US-assisted treatment (visual aspect). A sample mass in the range of 50–75 mg was suitable to be US-assisted digested under optimized conditions.

Another evaluated variable was the effect of performing the US-assisted digestion using tightly closed vessels, which increases the pressure inside the vessel and then special attention is required to open the glass vessels, or just covered glass vessels (not tightly closed). First, the residual carbon content (RCC, wt. %) of the digestates after the US-assisted digestions, using covered or tightly closed vessels. The analysis of RCC in the digestates is especially relevant when stripping voltammetric techniques are used for trace metal determinations because this technique can be affected by residual organic molecules present in the analyzed solution [2,20,27]. The RCC in the digestates generated after the application of the optimized US-assisted digestion, using covered or tightly closed vessels, on biodiesels produced from different oil sources is presented in Table 3.

INSERT TABLE 3

Interestingly, the RCC values obtained in the digestates generated after the US-assisted digestion using tightly closed vessels were higher in almost all cases. However, the RCC values obtained when digestions were performed in tightly closed vessels did not surpass 10% in relation to the other procedure. Then, these results indicated that the glass vessels did not require to be tightly closed for the US-assisted digestion of biodiesel samples. Table 4 summarizes the optimized conditions of the US-assisted digestion of biodiesel samples within the studied conditions.

INSERT TABLE 4

Another important parameter to be evaluated is the recovery values of metals after the US-assisted digestion. Table 5 presents the recovery values for a biodiesel sample (obtained from soybean oil) spiked with five metals using covered vessels. The determination of cadmium, copper, lead and zinc was performed simultaneously at a single scan using SWASV measurements, which presented superior performance (higher sensitivity) for metal determination in the digestates than stripping chronopotentiometry. Mercury determination in the digestates was carried out by SWASV using the Au-SPE in the BIA system. Table 5 also presents the concentrations found for each metal in this biodiesel sample before spiking. Figure 2 presents SWASV measurements for metal determination in this digestate obtained after US-assisted digestion and the respective analytical curves for Zn, Pb, Cu and Cd. Figure 3 presents SWASV measurements for Hg determination in the same digestate.

INSERT FIGURE 2 and FIGURE 3

INSERT TABLE 5

Recovery values between 94 and 108% were obtained, which attested the US-assisted digestion procedure. Similar recovery values (> 90%) were obtained using tightly closed vessels (results not shown). Therefore, these results also indicate that the glass vessels did not require to be tightly closed for the US-assisted digestion of biodiesel samples. Additionally, the recovery test demonstrated that the proposed US-assisted method can be applied for biodiesel samples in which volatile elements are required to be determined, such as mercury. The use of Au-SPEs cured at low temperatures (BT) for Hg determinations was preferred in this work because a previous work have demonstrated that this type of Au-SPEs presented higher electrochemical area due to the higher roughness in comparison with Au-SPE cured at high temperatures (AT) [28]. The high concentration of Zn in this biodiesel is due to its storage in galvanized steels that contain a protective zinc coating, which releases zinc due to corrosion processes [34].

All biodiesel samples were analyzed by SWASV after the US-assisted digestion using covered glass vessels. The concentration of all metals were below the respective LD values for the biodiesels obtained from colza, corn, cottonseed, and sunflower oils. An explanation for this result is that these biodiesels were produced from vegetable oils commercially available, which passes through a refining and purification processes. The concentrations of zinc found in the biodiesels produced from residual cooking oil and tallow were, respectively, 50 and 186 µg kg^{-1}. The content of cadmium, lead, copper and mercury were below the respective LD values also in these two biodiesels.

The efficiency of the proposed US-assisted digestion method was compared with other sample preparation methods reported for oil samples, which presents similar sample matrices, based on RCC values. Table 6 presents the RCC values for acid digestion of different oil samples, including the digested sample mass.

INSERT TABLE 6

The RCC values in the digestates obtained by the proposed US-assisted digestion were situated within the minimum and maximum values reported in other works reported in the literature for similar samples.

Most methods reported for oil samples applied microwave ovens to accelerate acid digestions and typical heating programs take 30–40 min plus additional time (20–30 min) to decrease the temperature of the flasks. The first works listed in Table 6 presented lower RCC values because a large amount of acids and oxidants were used in order to obtain final digestates free of organic compounds, which may interfere on stripping voltammetric analysis [20,27]. On the other hand, the proposed US-assisted method takes 40 min at room temperature, so it is not required any additional step to decrease the temperature. However, the digested sample mass was limited to 50-75 mg in order to obtain digestates free of residual sample matrix visually identified. Moreover, ultrasonic baths can be battery-powered [35] and the combination of the proposed US-assisted method with electrochemical stripping analysis using SPEs was demonstrated as possible analytical method for on-site metal determination in biodiesels. Similar combination has been recently reported for metal determinations in indoor dust samples [36-38].

Conclusions

A simple and effective sample preparation protocol for biodiesel samples based on the US-assisted digestion is demonstrated. The proposed method requires a simple ultrasonic bath that can treat dozen of samples at once, exploring the optimal region over the piezoelectric crystals of the bath and can applied for biodiesels obtained from different oil sources. The digestates presented RCC values in the range of 3.05 – 3.75%, which is comparable to values reported for digestates generated with the aid of microwave ovens. Recovery values of 94-108% demonstrated the accuracy of the proposed sample preparation method applied for the stripping voltammetric determination of metals in biodiesel and indicated that no losses of mercury occurs. The accuracy of voltammetric determinations can be affected by residual organic compounds in solution, thus it can be assumed that the proposed US-assisted digestion protocol is adequate for metal determinations by voltammetry and probably applicable to atomic spectrometric analyses. It is also demonstrated that the proposed US-assisted method for biodiesel samples can be associated with on-site electrochemical analysis using disposable screen-printed electrodes.

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FIGURE CAPTIONS

Figure 1. Contour lines of response surface plot for factorial design experiments to optimize (A) the volumes of concentrated HCl and H_2O_2 and (B) temperature of the bath and sonication time.

Figure 2. (Right) SWASV recordings for the determination of Zn, Cd, Pb and Cu in a biodiesel sample after US-assisted digestion under optimized conditions. Additions of (a) digested sample and six consecutive additions (b-g) of standard solutions containing 75 µg L^{-1} Zn, 50 µg L^{-1} Cd, and 25 µg L^{-1} Pb and Cu. (Left) the respective analytical curves for Zn (R = 0.997), Cd (R = 0.997), Pb (R = 0.997), and Cu (R = 0.996). SWASV experimental conditions as in Table 1.

Figure 3. SWASV recordings for the determination of Hg in a biodiesel sample after US-assisted digestion under optimized conditions. Additions of (a) digested sample and four consecutive additions (b-e) of standard solutions containing 20 µg L^{-1} Hg. Inset: the respective analytical curve (R = 0.991). SWASV experimental conditions as in Table 1.

TABLES

Table 1. SWASV parameters used for the determination of Zn, Cd, Pb and Cu at a mercury-film electrode (MFE) and of Hg at Au-SPE in digested samples of biodiesel.

Technical Parameters (unit)	**MFE**	**Au-SPE**
Conditioning potential (V)	0.0	0.55
Conditioning time (s)	20	90
Deposition potential (V)	–1.4	–0.55
Deposition time (s)	30	90
Equilibration time (s)	5	10
Frequency (Hz)	20	20
Initial potential (V)	–1.4	0.25
End potential (V)	0.0	0.60
Step (V)	0.008	0.004
Amplitude (V)	0.025	0.030

Table 2. Factors and levels used in the two factorial design experiments to optimize (A) volumes of HCl and H_2O_2 and (B) temperature of the bath and sonication time.

Experiment	**Levels Design**		**Factorial A**		**Factorial B**	
			V HCl (mL)	**V H_2O_2 (mL)**	**Time (min)**	**Temperature (°C)**
1	-1.0	-1.0	2.0	2.0	20	20
2	1.0	-1.0	4.0	2.0	40	20
3	-1.0	1.0	2.0	4.0	20	40
4	1.0	1.0	4.0	4.0	40	40
5	0.0	0.0	3.0	3.0	30	30
6	0.0	0.0	3.0	3.0	30	30
7	0.0	0.0	3.0	3.0	30	30
8	-1.44	0.0	1.56	3.0	15.6	30
9	1.44	0.0	4.44	3.0	44.4	30
10	0.0	-1.44	3.0	1.56	30	15.6
11	0.0	1.44	3.0	4.44	30	44.4

Table 3. Residual carbon content found after US-assisted digestion of biodiesel samples (50 mg) produced from different oil sources: tallow, colza, sunflower, corn, cottonseed, and residual cooking oil.

Biodiesel samples	**Residual Carbon Content (wt. %)**	
	Covered Vessel	*Tightly Closed Vessel*
Tallow	3.31	3.61
Colza	3.75	4.02
Sunflower	3.63	3.91
Corn	3.50	3.76
Residual cooking oil	3.59	2.97
Cottonseed	3.05	3.40

Table 4. Optimized conditions for the US-assisted digestion of biodiesel samples.

Digestion parameters (unit)	**Variation**	**Optimized value**
Volume de HCl (mL)	2.0 – 4.0	3.0
Volume de H_2O_2 (mL)	2.0 – 4.0	4.5
Time (min)	20 – 40	30
Temperature (°C)	20 – 40	20
Sample weight (mg)	50 – 250	50
Vessel	tightly closed - covered	covered

Table 5. Recovery values for zinc, cadmium, lead, copper, and mercury spiked in the methyl soybean biodiesel after US-assisted digestion using covered glass vessels (n=3).

Metals	**Concentration (μg Kg^{-1})**			**Recovery / %**
	Found	*Spiked*	*Recovery*	
Zinc	978	250	1228	108 ± 4
Cadmium	103	85	188	105 ± 5
Lead	70	51	121	107 ± 4
Copper	21	85	106	94 ± 5
Mercury	< LD	20	020	100 ± 10

Table 6. Residual carbon content (wt. %) in the digestates obtained after digestion of organic samples following different procedures reported in the literature.

Sample	Mass (g)	Method	RCC (wt. %)	References
Crude oil	0.1 – 0.85	MW-assisted	< 0.03	[20]
Diesel oil	0.1 – 0.85	MW-assisted	< 0.03	[20]
Lubricating oil	0.1 – 0.85	MW-assisted	< 0.03	[27]
Lubricating oil	0.1 – 0.2	MW-assisted	4.1 – 18.4	[29]
Lubricating oil	0.6	MW-assisted	2.37 – 9.71	[30]
Lubricating oil	0.5	MW-assisted	1.60	[31]
Lubricating oil +biodiesel (1:4)	0.5	MW-assisted	1.74	[31]
Biodiesel	0.5	MW-assisted	0.93	[31]
Diesel oil	1.5 – 3.0	MW-assisted	0.3 – 6.5	[32]
Soybean	0.25	MW-assisted	3.99 – 14.1	[33]
Biodiesel	0.05	US-assisted	3.05 – 3.75	This paper

MW-assisted: digestions carried out in microwave oven.

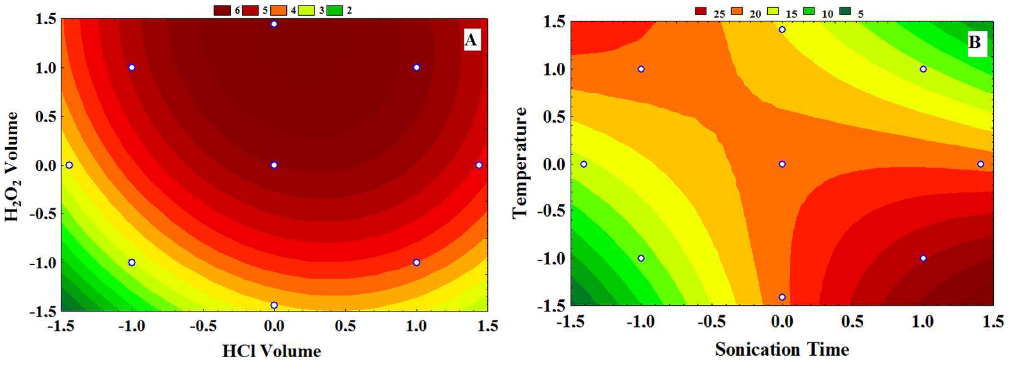

Figure 1. Contour lines of response surface plot for factorial design experiments to optimize (A) the volumes of concentrated HCl and H2O2 and (B) temperature of the bath and sonication time.
2412x850mm (72 x 72 DPI)

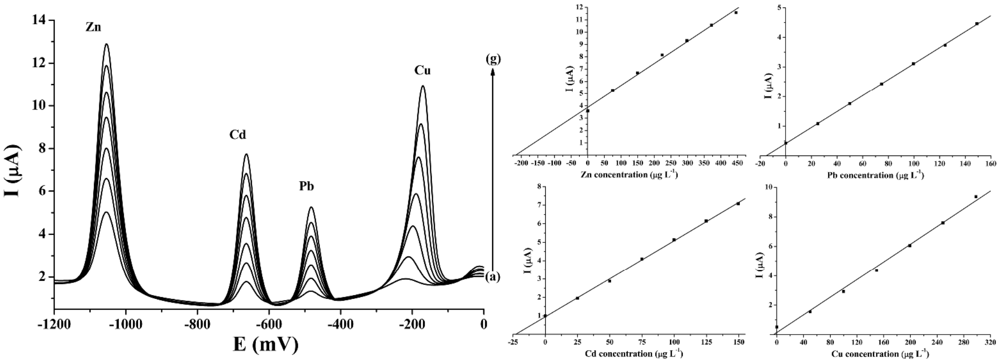

Figure 2. (Right) SWASV recordings for the determination of Zn, Cd, Pb and Cu in a biodiesel sample after US-assisted digestion under optimized conditions. Additions of (a) digested sample and six consecutive additions (b-g) of standard solutions containing 75 μg L-1 Zn, 50 μg L-1 Cd, and 25 μg L-1 Pb and Cu. (Left) the respective analytical curves for Zn (R = 0.997), Cd (R = 0.997), Pb (R = 0.997), and Cu (R = 0.996). SWASV experimental conditions as in Table 1.
705x253mm (300 x 300 DPI)

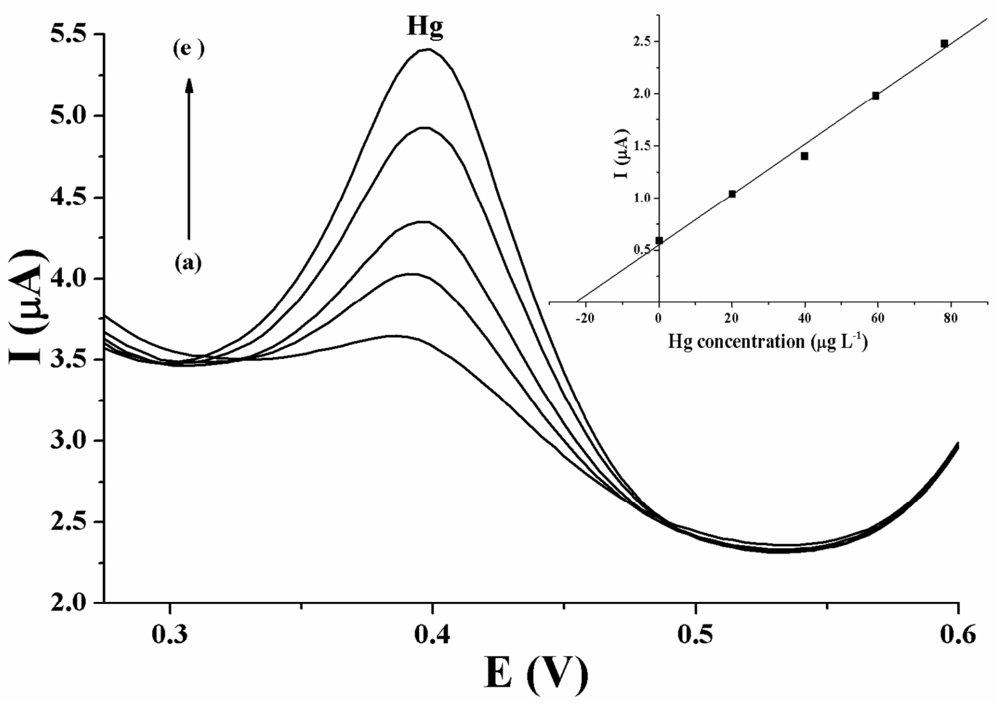

Figure 3. SWASV recordings for the determination of Hg in a biodiesel sample after US-assisted digestion under optimized conditions. Additions of (a) digested sample and four consecutive additions (b-e) of standard solutions containing 20 µg L-1 Hg. Inset: the respective analytical curve (R = 0.991). SWASV experimental conditions as in Table 1.
276x194mm (150 x 150 DPI)