



A simple and economical ultrasound-assisted method for Cd and Pb extraction from fruits and vegetables for food safety assurance

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ARTICLE INFO

Keywords:

Ultrasound-assisted extraction

Food safety

Confocal optical microscopy

ABSTRACT

Ultrasound-assisted extraction (UAE) methods for food analysis are recognized as being in accordance with the principles of green analytical chemistry (GAC). Reference standard methods generally use microwave-assisted extraction (MAE) for sample digestion; however, the use of ultrasonication processes has many advantages. Herein, a simple, and economical method for the determination of Cd and Pb in fruits and vegetables for food safety monitoring is reported. This method was optimized using a standard reference material (SRM, spinach leaves) through a multivariate experimental design (optimal conditions: 0.5 g of sample, 15 min of sonication, and 20.00 mL of 2.5 mol L⁻¹ HNO₃) and subsequently validated. The limits of detection obtained were one or two orders of magnitude lower than those achieved with MAE, making this a promising technique for contaminants monitoring. The UAE method was then tested on vegetable samples (apples, carrots, tomatoes, and lettuce) obtained from commercial sources in Uruguay and a comparison was made with samples treated by MAE. Both processes yielded comparable results, and the levels of Cd and Pb in all samples were below the maximum allowed concentration (MAC) established by the World Health Organization (WHO). In addition, to provide insight into the mechanism by which the ultrasonic process aids extraction, confocal optical images of the surface of the SRM were obtained. The leaves were examined after treatment at different sonication times (5–20 min). The confocal microscopy images show the effects of the ultrasonic treatment on the plant tissue, including desaturation, erosion, and abrasion, which increased with increasing sonication time. In conclusion, the UAE method meets and overcomes the requirements of a standard method for food safety assurance.

1. Introduction

Food safety is a matter of global concern, and policies and processes to ensure food safety must cover the entire food chain, from production to consumption. Safety is essential to ensure public health and is a crucial factor in commercial food production [1].

Fruits and vegetables are an important source of essential micronutrients, but there is growing concern about the accumulation of potentially toxic metals in these foods. Therefore, the monitoring of contaminants is crucial to assess health risks, and knowledge of the food composition is necessary to define food quality [2–4].

Cadmium reaches the soils and water via the indiscriminate use of phosphate fertilizers, which contain this element as a byproduct of their production. The accumulation of Cd-containing phosphate fertilizers in soils allows Cd to be absorbed by plants and, thus, incorporated into foods destined for human consumption [1,5]. Pb is naturally present in the environment. However, when there are high levels of Pb, this is typically a result of human activities. Cd and Pb are toxic metals, mainly affecting the kidneys and the cardiovascular and nervous systems of children and adults [6].

The increasing global awareness for food safety and contamination by chemicals related to human health and specially in low income

Abbreviations: UAE, ultrasound-assisted extraction; SRM, standard reference material; MAE, microwave-assisted extraction; GAC, green analytical chemistry; LOD, limit of detection; LOQ, limit of quantification.

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<https://doi.org/10.1016/j.rechem.2020.100089>

Received 12 October 2020; Accepted 16 December 2020

countries leads to the development of new safe, reliable, and economical analytical methods.

Ultrasonic techniques have numerous applications for food processing [7–9]. Although many ultrasound-assisted extraction (UAE) analytical methods have been reported, these are not standard methods for food analysis, and, so far, they have not been included in any international compendia such as the AOAC [7–17].

UAE methods have numerous advantages over other widely used methods, such as microwave-assisted extraction (MAE), for the determination of the mineral composition of foodstuffs. UAE is simple and accurate, and the effect of UAE on food has been characterized, thus explaining the success of this technique [9–16]. Thus, sonochemistry and analytical chemistry are good partners for food composition analysis. In addition to the advantages of UAE mentioned above, it is important to increase awareness of analytical methods that are consistent with the principles of green analytical chemistry (GAC) [18–20]. Cairós et al. discussed the use of sonochemistry in analytical chemistry and stated, “the secret relies on the bubbles,” in reference to the powerful effects of cavitation in sonicated liquids [20].

A useful tool to understand the processes occurring when food is subjected to sonication under set conditions is confocal microscopy [9,21]. Optical confocal microscopy images provide fine details and better contrast than conventional microscopy [22].

In this study, optical confocal microscopy images of a standard reference material (SRM; in this case, spinach leaves) were obtained to observe the physical effects of acoustic cavitation over the surface of the vegetable matter.

Thus, a UAE analytical method for the determination of Cd and Pb in apples, carrots, lettuce, and tomatoes was developed and validated. These fruits and vegetables were selected considering that they can be eaten raw and its consumption may involve higher risks. Multivariate experiments were performed for optimization. The proposed UAE method meets the requirements established by the AOAC for standard methods for contaminant analysis. In addition, the UAE method has several advantages compared with the traditional MAE method.

2. Materials and methods

2.1. Reagents

Calibration curves were obtained using atomic absorption spectrometry. Solutions of appropriate dilution were prepared in ultrapure water using commercial standard solutions containing 1000 mg L⁻¹ of Cd and Pb (Merck, Darmstadt, Germany). Commercial solutions of palladium nitrate (Pd(NO₃)₂) (10,000 mg L⁻¹, Merck, Darmstadt, Germany) and ammonium phosphate monobasic (NH₄H₂PO₄) (5000 mg L⁻¹, Sigma-Aldrich, St. Louis, USA) were used to prepare chemical matrix modifiers for Cd and Pb determination. All glassware was soaked overnight in 10% v/v HNO₃ prepared from 67% w/w HNO₃ (Merck, Darmstadt, Germany) and then rinsed exhaustively with ultrapure water. Ultrapure water having a resistivity of 18.2 MΩ.cm (ASTM Type I) was obtained from a water purification system (Millipore DirectQ3 UV, Darmstadt, Germany). Other reagents used were all analytical grade.

2.2. Samples

Samples of fresh fruits and vegetables were obtained from the *Mercado Modelo*, the central municipal market in Montevideo, Uruguay. A random selection of five sellers was obtained following a protocol, established by the municipal safety authority, where each sample was representative of the total sale volume of the day. Sampling was carried out during the first hours of the market's working hours (6:00 to 10:00 AM) and the samples were sent to the laboratory and processed the same day.

The selected fruits and vegetables for this study were lettuce, tomatoes, carrots, and apples. In the case of lettuce, five units from different sellers were randomly selected to obtain several pools of lettuce samples. For apples, tomatoes, and carrots, samples of at least 1 kg of the fresh vegetables were obtained following the same randomization protocol. The samples were packed in virgin polyethylene bags, sealed, coded, and transported to the laboratory where they were quartered and sub-sampled in portions of 100–200 g. This was carried out on the same day as sampling. Then, the samples were cut with a ceramic knife on a plastic surface and dried in a forced circulation stove DN93 (Yamato, Tokyo, Japan) at 70 °C for 96 h. Once dried, they were processed in a hammer mill (Willey Model 4, Thomas Scientific, NJ, USA), sieved through 1-mm sieve, and kept in conical plastic tubes under low-humidity conditions until analysis.

A spinach leaf SRM (NIST 1570a, Gaithersburg, MD, USA) was used for the optimization and validation of the analytical method.

2.3. Sample preparation procedures

2.3.1. Proposed UAE method

An ultrasonic homogenizer (Sonics™ Vibracell, 750 W; 20 kHz; 230 VAC, USA) equipped with a 13-mm titanium alloy probe was used.

The sample preparation procedure was as follows: 0.5 g of dry powder was accurately weighed in a 50-mL polypropylene vessel and then, 20.00 mL of 2.5 mol L⁻¹ HNO₃ was added. The ultrasound probe was immersed in the mixture for 15 min at a sonication amplitude of 35%. Once the treatment had finished, the obtained suspension was centrifuged for 15 min at 28,000g, and the resulting supernatant was used for analysis. Each sample was analyzed in triplicate, and reagent blanks were also run.

2.3.2. MAE reference method for total digestion

For comparison of the proposed UAE method with a sample preparation procedure based on total digestion, microwave-assisted extraction (MAE) in acidic media was performed using the same samples. For this purpose, a microwave oven (CEM, Mars 6, North Carolina, USA) with 12-vessel capacity (Easy Prep Plus, North Carolina, USA) was employed. In this procedure, 0.5 g of the dry powder sample was accurately weighed in the microwave reaction vessels and then, 10.00 mL of 4.8 mol L⁻¹ HNO₃ was added [18]. The applied program was: 15-min ramp until 200 °C and then, 10 min at 200 °C. The power varied between 400 and 1800 W, and the maximum pressure was 500 psi. Finally, the samples were allowed to cool for 15 min. Once the digestion had completed, the obtained solutions were quantitatively transferred, and the samples were made up to a final volume of 20.00 mL with ultrapure water. These solutions were used for the analytical determination of Cd and Pb. All samples were analyzed in triplicate, and reagent blanks were also run.

2.4. Analytical determination

Analytical determination of Cd and Pb was achieved using electrothermal atomic absorption spectrometry (ETAAS) using a spectrometer with Zeeman background correction (Thermo Scientific iCE 3500, Cambridge, UK), a transversely heated graphite-tube furnace module (Thermo Scientific GFS35Z, Cambridge, UK), and an autosampler module (Thermo Scientific GFS33, Cambridge, UK). The analytical lines employed were 228.8 nm (Cd) and 283.3 nm (Pb), and the signal used for quantification was the integrated absorbance (peak area). Pyrolytically coated graphite tubes were provided by Thermo Scientific, and the purge and protective gas was argon (99.998%, Praxair, Uruguay). The graphite furnace heating programs used for the analytical determinations are presented in Table 1. The injection volume was 20 µL in all cases. The chemical matrix modifier used for Cd determination consisted of 10 µL of a solution containing 5 µg of Pd(NO₃)₂, whereas, for Pb, this was 10 µL of a solution containing 2.5 µg of Pd

Table 1

Experimental conditions. Temperature programs for Cd and Pb determinations by ETAAS.

Stage	Temperature (°C)	Hold time (s)	Ramp rate (°C s ⁻¹)
Drying	110 ^(Cd) /120 ^(Pb)	20 ^(Cd) / 35 ^(Pb)	10
Pyrolysis	800 ^(Cd) /900 ^(Pb)	20	150
Atomization	1200 ^{*(Cd)} /1800 ^{***(Cd)} /1200 ^{*(Pb)} / 1400 ^{***(Pb)}	3 ^(Cd) /4 ^(Pb)	0
Cleaning	2500	3	0

* Atomization temperature for the UAE method. ** Atomization temperature for the MAE method.

(NO₃)₂ and 25 µg of NH₄H₂PO₄. In all cases, the argon gas flow rate was 0.2 L min⁻¹ at all stages (except for atomization).

2.5. Optimization and validation

For the optimization of the proposed UAE method, several critical variables were evaluated. For this method, the most critical variables that influence the analyte extraction process are the concentration of the extractant (dilute nitric acid) and sonication time. The effects of these variables were studied by means of a multivariate experimental design. The optimal experimental conditions were obtained by means of a two-level central composite design in which the HNO₃ concentration (1–3.5 mol L⁻¹) and sonication time (5–20 min) were varied. The spinach leaf SRM (NIST 1570a) was selected for optimization and validation because of its similarity with the matrices under study and because it contains quantifiable levels of Cd and Pb. This SRM is widely used not only for the validation of new methods but also for quality control in food analysis [17]. Surface responses were obtained using Minitab™.

After optimizing the sample preparation stage, the methodology was validated following the Eurachem Guide recommendations [23]. The figures of merit evaluated were linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and trueness. Linearity was studied by constructing a six-point calibration curve (each level in triplicate). The LOD was estimated using a signal-to-noise ratio of 3 (3 s criterion, $n = 10$), and, analogously, the LOQ was determined using a signal-to-noise ratio of 10 (10 s criterion) [23]. Precision is expressed as relative standard deviation (RSD, %) after the analysis of six replicates of the SRM and with real samples. Trueness was evaluated by statistical comparison of the values obtained using the UAE method for the analysis of the SRM of spinach leaves with the corresponding certified values of Cd and Pb (percentage recovery (R%)) and for real samples by comparison with the results obtained using the MAE method (total digestion). In both cases Student's *t*-test was used [24].

2.6. Confocal microscopy

Confocal microscopy characterization was performed utilizing a WITec Alpha 300RA confocal Raman microscope. A small amount of the spinach leaf SRM without previous treatment or solid residues obtained after different sonication times were deposited on a glass substrate for optical imaging in air (5x and 100x magnification). Raman spectra could not be measured because of intrinsic fluorescence, which could not be avoided even using two different excitation laser wavelengths (532 and 785 nm). Nevertheless, the purpose of this part is to provide optical characterization with confocal resolution.

3. Results and discussion

3.1. Optimization and validation

3.1.1. Optimization

One of the objectives of this work is to develop a simple UAE method for the simultaneous determination of two inorganic contaminants in foodstuffs. Here, we focus on the optimization of the process. The percentage recovery (R%), defined as the ratio between the obtained results and the correspondent certified value of the SRM (NIST 1570a), was used as the response to optimize. The goal was to achieve an R% value close to 100 using the simplest conditions required to determine Cd and Pb with the same procedure and complying with standard analytical requirements. The Official Methods of Analysis of AOAC International is the most widely compendium of standard methods used in food analysis; therefore, we critically analyzed the developed UAE method against the AOAC requirements [17].

The results obtained after performing the experimental design are summarized in Table 2, and the effects of critical variables are shown in the response surfaces in Fig. 1. Table 2 also lists the final temperature of the suspension because an increase in temperature can damage vegetal structures, mainly proteins, releasing metals into the extracting solution [25]. Thus, the combination of the sonication, acid medium, and *in situ* heating contribute to the quantitative extraction of Cd and Pb.

According to the AOAC, the R% value using a suitable SRM must range from 60% to 115%. For our target contaminants, an R% at the lower end of this range is adequate because they are typically present at the trace level [17,26]. For the selected vegetables, the NIST-provided SRM is appropriate and widely used for this type of validation.

Experiments 2–4 and 7–9 did not yield adequate recoveries for both Cd and Pb, whereas experiments 1 and 6 do meet the standards for the recoveries of both Pb and Cd. However, note that some other experiments showed better individual recoveries for Cd or Pb. Experiment 5 showed the best performance for both elements, having a R% for both elements meeting the AOAC requirements, and the combination of the use of dilute acid and moderate sonication time is favorable; thus, the selected optimum conditions for the method validation are 2.5 M HNO₃ and 15 min of sonication. Under these conditions, the final temperature caused by bubble collapse during cavitation was 82 °C, one of the highest temperatures reached in this experimental design.

3.1.2. Validation

The UAE method was validated for Cd and Pb determination in apple, lettuce, carrot, and tomato samples according to the recommendations of the Eurachem Guide. Validation was carried out with respect to the main figures of merit, as explained in Section 2.5 [23]. Table 3

Table 2

Results corresponding to the multivariate experimental design [21].

Experiment	HNO ₃ (mol L ⁻¹)	Time (min)	Cd (R%)	Pb (R%)	Final T (°C)
1	2.5	5	95 ± 4	63 ± 3	70
2	2	10	54 ± 6	94 ± 3	70
3	3	10	50 ± 3	108 ± 2	78
4	1	15	66 ± 4	125 ± 2	76
5	2.5	15	81 ± 3	112 ± 5	82
6	3.5	15	64 ± 4	74 ± 4	86
7	3	20	60 ± 3	55 ± 10	88
8	2	20	59 ± 3	154 ± 5	76
9	2.5	20	72 ± 7	51 ± 4	77

R%: percentage recovery using the SRM (NIST 1570a). Mean ± standard deviation ($n = 3$).

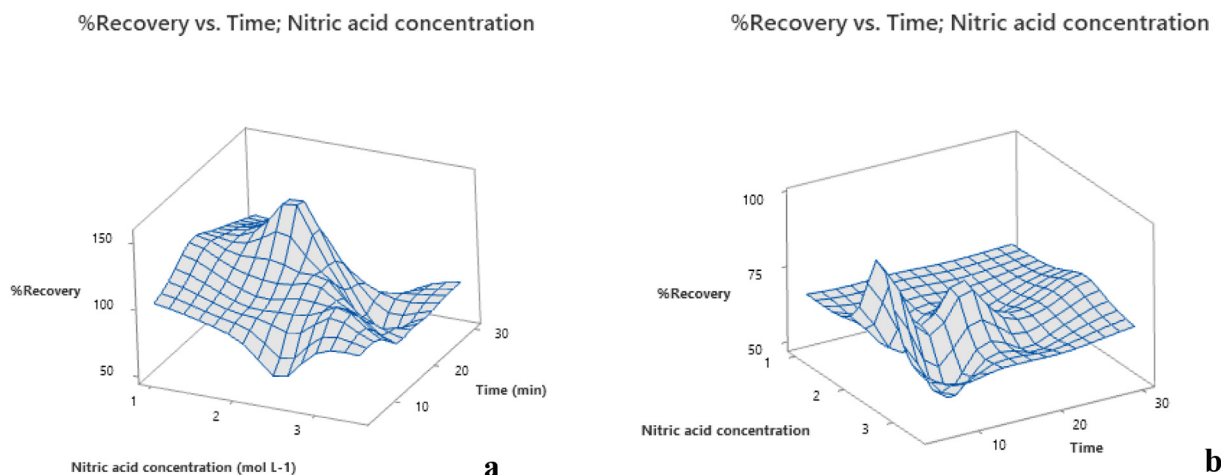


Fig. 1. Response surface obtained for Cd (a) and Pb (b).

Table 3

Figures of merit for UAE and MAE.

	UAE: Cd/Pb				MAE: Cd/Pb			
	LOD	LOQ	Trueness* (R%)	RSD, %	LOD	LOQ	Trueness* (R%)	RSD, %
Lettuce	0.07/0.7	0.2/2.1	112 ± 7/97 ± 9	9.3/5.3	0.4/1.0	1.0/3.0	96 ± 8/111 ± 8	15/15
Tomato	0.3/0.7	1.0/2.1		15/3.4	0.5/3.3	2.0/11		7.8/12
Apple	0.2/1.2	0.6/4.1		6.2/7.1	1.2/2.5	4.2/8.4		8.5/6.7
Carrot	0.2/0.4	0.7/1.4		12/6.4	2.2/3.1	7.3/10		10/11

LOD and LOQ expressed in $\mu\text{g kg}^{-1}$ fresh weight. * Mean $\pm$ standard deviation ($n = 6$) using SRM (NIST 1570a) [23].

summarizes the validation results for the UAE method, as well as the results obtained using the MAE method for comparison. Good linearity was observed for both elements, having coefficients of determination (R^2) greater than 0.999. Individual residuals were also studied, and their random distribution was verified [27].

The maximum allowable concentration (MAC) for Cd and Pb according to regional regulations agreed by the Mercosur trade bloc and the Codex Alimentarius Commission are 0.2 and 0.3 mg kg^{-1} (expressed in fresh weight), respectively, in leafy vegetables and 0.1 mg kg^{-1} of Cd and Pb in fruits and other vegetables [28,29]. Notably, the LODs and LOQs (see Table 3) obtained with the UAE method expressed in micrograms/kilogram of fresh weight were one and two orders of magnitude below the MAC, respectively, so the detectability is more than enough for food safety monitoring. These results also comply with the requirements of the AOAC, that is, an LOD at least 10 times lower than the MAC. Thus, the UAE method could be considered as a standard method for the analysis of these contaminants in food [17]. In particular, the LODs are considerably lower (10 times)

than those reported by Machado et al. for both elements in other vegetables, and the acid used as an extractant is 1.4 times more dilute [9].

For trueness evaluation, the experimental t -values obtained by means of Student's t test were 2.40 and -0.72 for Cd and Pb, respectively, both below the theoretical value ($t(0.05, 6) = 2.57$), indicating that, at the 95% confidence level, the obtained concentrations of both elements did not differ significantly from the certified values of the SRM. The precision in all cases was better than 15% (RSD). This is adequate considering that these elements, when detected, are at the trace concentration level and is in accordance with the provisions of AOAC (RSD < 15%) for both elements in the four studied matrices [17].

To compare the performance of UAE with classical MAE, figures of merit for MAE were also obtained, and the results are listed in Table 3. The LOD and LOQ values obtained using the UAE method are significantly lower than those obtained using MAE, which is related to the high precision of the UAE process. This is advantageous for trace element determination, particularly when applied to monitor inorganic contaminants because, in addition to foods containing contaminants

Table 4

Cd and Pb levels in samples. Comparison UAE and MAE.

	UAE	MAE	UAE	MAE
	Cd (mg kg^{-1})		Pb (mg kg^{-1})	
Lettuce	0.013 $\pm$ 0.002	0.016 $\pm$ 0.004	0.0017 $\pm$ 0.0002	0.0017 $\pm$ 0.0001
	0.035 $\pm$ 0.002	0.030 $\pm$ 0.002	0.0047 $\pm$ 0.0005	0.0041 $\pm$ 0.0001
	0.048 $\pm$ 0.002	0.044 $\pm$ 0.002	0.0021 $\pm$ 0.0005	0.0029 $\pm$ 0.0006
Carrot	<0.0007	<0.007	<0.0014	ND
Tomato	ND	ND	<0.0021	ND
	<0.0010	<0.0020	<0.0021	ND
Apple	0.019 $\pm$ 0.004	0.017 $\pm$ 0.003	0.0019 $\pm$ 0.004	0.017 $\pm$ 0.003
	<0.0006	ND	0.040 $\pm$ 0.023	0.044 $\pm$ 0.002

Mean $\pm$ standard deviation ($n = 3$). Fresh weight. ND = not detected.

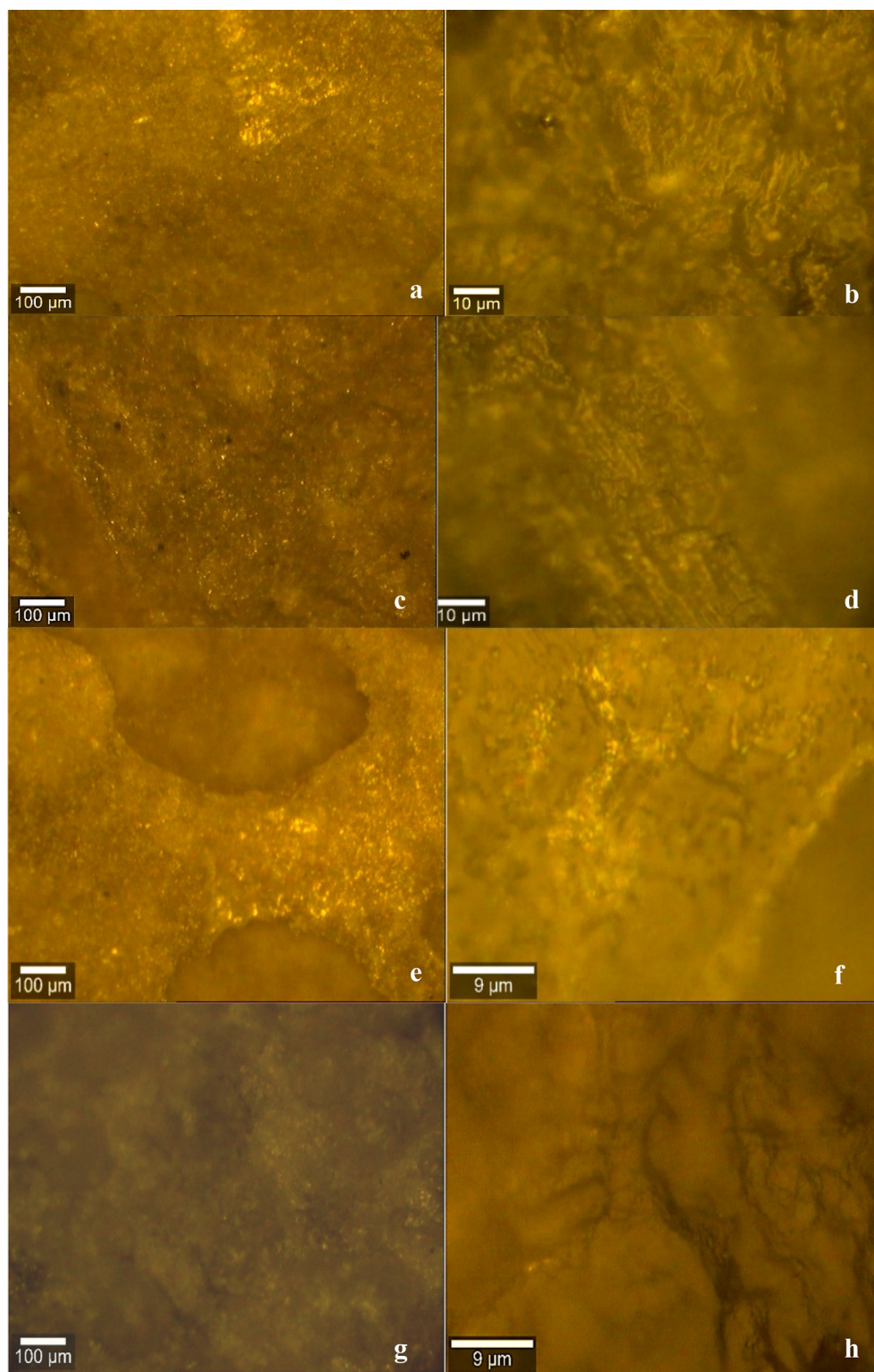


Fig. 2. Confocal optical images of a control sample (SRM NIST 1570a) without treatment (a, b); the SRM sample after 5-min sonication (c, d); after 15-min sonication (e, f) and after 20-min sonication (g, h). Scale bars are 100 and 10 μm , respectively.

below the MAC, more information can be obtained about the levels of these contaminants for preventive purposes. However, when an ultrasound probe is used, only one sample at a time can be processed, whereas several can be treated simultaneously in a microwave oven. Nevertheless, the UAE process lasts 30 min, yielding a solution ready for measurement, whereas, for the MAE, the digestion program lasts

45 min and, subsequently, each sample must be individually quantitatively transferred and made up to the final volume by the analyst.

Several factors favor UAE as a green analytical method, including the use of dilute acid, the simultaneous determination of Cd and Pb, simple operation without the need for a microwave oven, and lower atomization temperatures required for ETAAS, which extends the life-

time of the graphite furnaces, thus making the process more economical. In addition, compared with those obtained with the MAE method, the LODs and LOQs are remarkably low, and all the figures of merit are in accordance with the AOAC requirements. Thus, this alternative method could yield better quality than standard methods.

3.2. Real sample analysis

Once the method had been validated, it was applied to local samples. Table 4 show the results obtained using UAE compared with those obtained using MAE to demonstrate the robustness of the former method. Results were expressed in fresh weigh. Moisture percentage of the raw vegetables were: 95.2%, 94.5%, 86.3% and 82.8% for lettuce, tomato, carrot, and apple, respectively.

Although several samples contained measurable levels of Cd and Pb, they were below the MAC values for both elements. Therefore, these food samples are considered safe for consumption.

The inorganic contaminants present in fruits and vegetables cannot be removed by washing or other treatment techniques because the elements are integrated in the plant tissues. Thus, if high levels of metals are found, the source of pollution must be identified and minimized. Consequently, it is crucial to determine the amounts of Pb and Cd, even though they are low, to identify risk factors, such as contaminated areas of cultivation. On comparing the UAE and MAE results, we noticed that not only are the results are comparable, which demonstrates robustness, but also, because of the low LOQs, more information about the Cd and Pb levels could be obtained, making this an accurate method for monitoring purposes.

3.3. Optical characterization by confocal microscopy

Confocal microscopy offers slightly higher resolution than conventional microscopy and, in the case of transparent samples, allows three-dimensional information to be obtained. Analysis is carried out by taking images at different depths to identify distinctive features of each sample [30]. The effects of treatments have been similarly analyzed and characterized using confocal microscopy as reported in previous work [9]. Thus, complementary information about the effect of the ultrasonication process on the spinach leaf SRM was obtained using this technique.

The SRM spinach leaves were used for the optimization and validation of the method, so confocal microscopy images were obtained of this sample without any treatment (control) and after different sonication times with constant concentrations of the extractant ($2.5 \text{ mol L}^{-1} \text{ HNO}_3$). The images were obtained at 5x and 100x magnification, allowing us to observe structures of approximately 100 and 10 μm (Fig. 2).

As shown by the images, the ultrasonic treatment resulted in the loss of the fibrous structure in the control (Fig. 2 a-h). This result is consistent with those described by Machado et al. [9]. The effect of ultrasonication is not drastic with $2.5 \text{ mol L}^{-1} \text{ HNO}_3$ and 5 min of sonication, whereas after 15 min the effects are significant i.e., the plant structures were damaged. Importantly, as shown in Section 3.1.1., extraction was efficient and quantitative for Cd and Pb after treatment for this time. After 20-min sonication, erosion, and abrasion over the surface of the vegetable can be observed in Fig. 2 (g-h).

The optimum sonication time was, thus, 15 min, and this treatment time was enough for the accurate determination of both elements. As Petigny et al. reported, there is an optimal sonication time that allows the extractant medium access to the substrate surfaces to allow erosion and extraction [31]. After 20-min sonication, the surfaces became smoother, and this was also observed by Chemat et al. in caraway seeds and reported as the detexturation of cell structures [8]. Thus, the images show changes on the surface of the spinach leaves consistent with detexturation, sonoporation, and erosion caused by ultrasonic waves. Therefore, confocal microscopy probed to be an

important technique for the structural characterization of ultrasonicated samples at a microscopic level.

4. Conclusions

An analytical method based on ultrasound-assisted extraction with dilute acid was developed for the determination of Cd and Pb in apples, tomatoes, carrots, and lettuce. This method is simpler, more economical, and more consistent with the principles of green analytical chemistry than those based on microwave-assisted digestion. The results of the validation showed that the ultrasonic method can be considered a reference standard method for food safety monitoring. In addition, via confocal microscopy, we verified several structural effects of sonication on the surfaces of SRM spinach leaves used for validation. Detexturation, erosion, and abrasion were evident when the sonication time was increased from 5 to 20 min at a constant concentration of extractant. Several vegetable and fruit samples from a Uruguayan market were analyzed using the developed method, and the concentrations of Cd and Pb were far below the corresponding maximum allowed concentrations, indicating their safety. The results of this work reinforce the utility and efficiency of ultrasound-assisted extraction methods applied in sample preparation procedures for the development of greener analytical methods and how promising they are to explore other matrices.

CRedit authorship contribution statement

Mónica Pereira: Data curation, Formal analysis, Investigation, Methodology, Validation. **Florencia Tissot:** Conceptualization, Data curation, Investigation, Methodology, Validation. **Ricardo Faccio:** Data curation, Investigation, Formal analysis. **Facundo Ibáñez:** Funding acquisition, Investigation, Project administration. **Mariela Pistón:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, 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.

Acknowledgements

Funding: This work was supported by the Agencia Nacional de Investigación e Innovación [ANII FSA_I_2017_1_140199], Comisión Sectorial de Investigación Científica (CSIC), and PEDECIBA-Química.

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