American Journal of Food Science and Technology, 2015, Vol. 3, No. 3, 55-59
Available online at http://pubs.sciepub.com/ajfst/3/3/1
© Science and Education Publishing
DOI:10.12691/ajfst-3-3-1

A Rapid Sample Preparation Method for the Determination of Cadmium and Lead in Spinach and Artichoke Leaves Using Ozone

Ignacio Machado[1], María Verónica Cesio[2], Isabel Dol[1], Mariela Pistón[1,*]

[1]Analytical Chemistry, Faculty of Chemistry, Universidad de la República, Montevideo, Uruguay
[2]Department of Natural Products and Pharmacognosy, Faculty of Chemistry, Universidad de la República, Montevideo, Uruguay
*Corresponding author: mpiston@fq.edu.uy

Received May 18, 2015; Revised June 03, 2015; Accepted June 07, 2015

Abstract A novel method for sample preparation using an ozone-based process was optimized for the determination of Cd and Pb using electrothermal atomic absorption spectrometry (ET-AAS). Advanced oxidation processes, which involve the *in situ* generation of highly potent chemical oxidants, are a promising technology to accelerate the oxidation at room temperature and the degradation of organic matter. A certified reference material of spinach leaves was used to evaluate the methodology. The sample was treated with diluted nitric acid, then a glass device was immersed in the solution and ozone was passed through for 10 minutes. The experimental conditions were optimized by means of a multivariate experiment. Figures of merit were: detection limits (3s): 0.002 mg kg^{-1} (Cd), 0.01 mg kg^{-1} (Pb), relative standard deviation lower than 10 % for both elements (n=5), the mean recovery was 97.6 % (Cd) and 98.3 % (Pb) compared with the certified value (n=5). The method was also applied for monitoring these elements in artichoke leaves and the results were in good agreement with those obtained with microwave digestion procedure. This procedure is simple, fast and showed to be adequate for the monitoring of these inorganic contaminants in spinach leaves and artichoke leaves.

Keywords: *ozone, cadmium, lead, spinach, artichoke, contaminants in food*

Cite This Article: Ignacio Machado, María Verónica Cesio, Isabel Dol, and Mariela Pistón, “A Rapid Sample Preparation Method for the Determination of Cadmium and Lead in Spinach and Artichoke Leaves Using Ozone.” *American Journal of Food Science and Technology* vol. 3, no. 3 (2015): 55-59. doi: 10.12691/ajfst-3-3-1.

1. Introduction

In the MERCOSUR region (acronym of the Southern Common Market) there exists a technical regulation on maximum limits of inorganic contaminants in food as well as in the European Union. This regulation must be fulfilled by the member countries of MERCOSUR (Argentina, Brazil, Paraguay and Uruguay). It establishes criteria for commercialization of food between these countries and those imported from elsewhere. The maximum limits allowed for cadmium (Cd) and lead (Pb) in vegetable edible leaves are 0.2 mg kg^{-1} and 0.3 mg kg^{-1} respectively [1].

To ensure food safety, the laboratories need simple and rapid validated analytical methodologies to provide fast response, to allow the commercialization of the products.

The official methods, although they have been reviewed and tend to decrease processing time of the samples, are based on acid digestions involving a long time; they have many stages and consume large amounts of dangerous reagents (mainly mineral acids to oxidize organic matter). Quite recently, the reference manuals began to incorporate microwave-assisted treatments in the standard methods; this proved to be an efficient strategy for extracting metals and sample preparation [2]. A microwave digestion equipment costs approximately USD 25.000 in South America, which is expensive for laboratories and has high-energy consumption.

Attention is currently given to certain critical parameters in the development of new methods, such as operating time, security, ratio of volume / concentration of solvents, energy consumption, among others [3].

Oxidation processes involving in situ generation of oxidant chemical species have been reported as effective emerging technologies to accelerate the degradation of organic matter, at room temperature, in certain matrices. These processes, commonly called “advanced oxidation processes” (AOPs), generally use ozone combined with UV radiation [4,5].

Ozone-based procedures are used to remove organic matter that, otherwise are very difficult to remove, e.g. from water or industrial effluents [6-8].

It is also used to remove organic contaminants in waters and polluting effluents emerging in the pharmaceutical industry using the advance of the oxidizing ability to degrade organic compounds and pesticide residues [9,10,11,12].

Only a few works reported that ozone gas (combined with the use of ultrasound and/or UV radiation) can be used for the sample preparation of matrices containing organic matter for the determination of metals and semimetals (ozonation and sonication) [5,6,8,13,14].

Khuntia et al. in 2014 postulated that ozone would be effective as an oxidant of organic matter due to the formation of hydroxyl radicals in situ [8], but there is scarce information regarding the possible mechanisms.

In this work the optimization and validation of a novel method for the determination of Cd and Pb in spinach and artichoke leaves with an ozone-based procedure for sample pre-treatment is presented. The entire method can be performed in 15 minutes using dilute acid. The analytical determinations were carried out by ET-AAS.

Artichoke leaves are widely used for infusions and for phytopharmaceutical products. An infusion preparation of artichoke leaves has recognized and demonstrated antioxidant capacity and high content of bioactive compounds [15].

The proposed procedure showed to be adequate for the purpose and could be a useful tool for the control of these inorganic contaminants in spinach and artichoke leaves and similar green leafy vegetables for food control. The treatment is simple, fast and also economical, with the novelty of the use of ozonation in the sample treatment. To the best of our knowledge, this has not been reported for Cd and Pb or for this matrix before.

2. Materials and Methods

2.1. Instrumentation

Ozone was generated from oxygen (99.5%, Linde, Montevideo, Uruguay) with a corona discharge ozone generator (OZOX - OG 75-A, Montevideo, Uruguay) with an oxygen flow rate of 7 L min-1. A borosilicate 3.3 glass (Pyrex®) piece, constructed by a glassblower at the Faculty, was placed at the end of the tube that transports the gas. This device has a porous glass membrane (pore size: 200 µm) through which ozone gas passes into the solution during the ozonation process.

For the analytical determinations of Cd and Pb an iCE 3500 Atomic Absorption Spectrometer with graphite furnace (Thermo Scientific, Cambridge, United Kingdom) was used. The light sources were hollow cathode lamps (Photron Pty. Ltd., Victoria, Australia), operated at the 228.8 nm (Cd) and 283.3 nm (Pb) analytical lines. The spectrometer was controlled with specific software SOLAAR (Thermo Scientific, Cambridge, United Kingdom). Integrated absorbance (peak-area) was used for signal evaluation and quantification. The GFS35Z transversely heated graphite tube furnace supplied with the iCE 3500, and the GFS33 auto sampler (both from Thermo Fisher Scientific) were used throughout. Injections were of 20µL sample solutions. Extended lifetime graphite tubes (Thermo Scientific) were used. Argon 99.998 % (Linde, Montevideo, Uruguay) was used as the purge and protective gas.

The graphite furnace heating programs used for the analytical determinations are showed in Table 1 where the optimized conditions are described for each element. The optimal temperatures for pyrolysis and atomization were 350/1000°C and 1500/1800 ºC for Cd/Pb respectively.

Chemical matrix modifier for Cd and Pb: 10 µL of solution containing 5 µg of $Pd(NO_3)_2$ and 3 µg of $Mg(NO_3)_2$. For Cd two drying steps were required [16].

Table 1. Heating programs for the determination of Cd and Pb using ET-AAS with Zeeman background correction

Stage	Temperature (ºC)	Ramp rate (ºC/s)	Hold time (s)	Ar flow rate (L/min)
Drying 1	100	$5^{Cd}/10^{Pb}$	30	0.2
Drying 2 ^{Cd}	140	15	20	0.2
Pyrolysis	$350^{Cd}/1000^{Pb}$	$10^{Cd}/150^{Pb}$	$0^{Cd}/20^{Pb}$	0.2
Atomization	$1500^{Cd}/1800^{Pb}$	0	3	0
Cleanup	2600	0	3	0.2

2.2. Reagents and Samples

All chemicals used were of analytical reagent grade. Ultrapure water of 18.2 MΩcm resistivity (ASTM Type I) was obtained from a Millipore Simplicity 185 purifier. All glassware was soaked overnight in 10% (v/v) nitric acid and then rinsed exhaustively with ultrapure water.

Nitric acid (HNO_3 65%, Merck, Germany) was used for sample preparation.

Stock standard solutions of Cd and Pb, containing 1000 mg L^{-1} (Merck, Darmstadt, Germany) were used to prepare the working standard solutions with concentrations 10 mg L^{-1} for Cd and Pb, in 0.1% w/w HNO_3. Working aqueous standard solutions were prepared fresh daily by dilution with 0.1% w/w HNO_3. The calibration curves for ET-AAS measurements were constructed in the range 0.3 - 4 µg L^{-1} for Cd and 2-50 µg L^{-1} for Pb. Stock solutions of $Pd(NO_3)_2$ (Merck, Darmstadt, Germany) and $Mg(NO_3)_2$ (Aldrich Chemical Company, Milwaukee, WI, USA) containing 10000 and 20000 mg L^{-1} respectively, were used to prepare the chemical matrix modifier. The chemical matrix modifier for Cd and Pb was a mixture containing 5 µg of $Pd(NO_3)_2$ and 3 µg of $Mg(NO_3)_2$ in a 10 µL volume, injected into the furnace with the auto sampler.

A certified reference material (CRM) of trace elements in spinach leaves (NIST 1570a) was used for the optimization and for the evaluation of the trueness and precision of the proposed method. This material consists of U.S. Grade A chopped frozen spinach. At NIST, the freeze-dried material was sieved through a polypropylene sieve having pores of 0.25 mm. The sieved material was then jet milled and air classified to a particle size of approximately 75 µm (200 mesh) [17].

The certified value for Cd was (2.876 ± 0.058) mg kg^{-1} (dry-mass basis). For Pb the informed value was 0.2 mg kg^{-1} (dry-mass basis); in this case, the certificate did not provide the uncertainty.

Artichoke leaves (2 kg) were collected in Montevideo, Uruguay. Professor Eduardo Alonso Paz (Curator of the Herbarium MVFQ, Uruguay) identified the fragments of leaves as *Cynara cardunculus* subsp. cardunculus. A voucher specimen (MVFQ 4399) has been deposited in the Herbarium of the Cathedra of Botany of the Faculty of Chemistry, Universidad de la República, Montevideo, Uruguay. The artichoke leaves were chopped and dried in an oven with forced air circulation (70 °C) and stored at 20 °C at light-free conditions. Before sample preparation, the material was milled in order to obtain a particle size similar to that of the CRM.

For the validation of the Cd and Pb determination in artichoke leaves a microwave assisted digestion was carried out using a MARS-6 (CEM, Mathews, NC, USA) equipment. The procedure for this digestion consisted in the digestion of the artichoke samples (0.3 g) with 10 mL HNO_3 65% and using a microwave program provided by the manufacturer called “Plant material”. This program has two stages with a maximum temperature of 200 °C [19].

2.3. Sample Preparation

To quantify ozone concentration, the iodometric method was used according to the APHA standard method [18]. Briefly, in this method, the ozone gas is bubbled into a solution of KI 20 g L^{-1}, and the amount of iodine formed is titrated with a standard solution of $Na_2S_2O_3$ (0.005 mol L^{-1}) using starch suspension as indicator. The ozone concentration in solution under the optimum experimental conditions was 14 mg L^{-1} (ozonation time: 10 minutes).

The sample was prepared as follows: 0.5 g were accurately weighed in a glass vessel and then 10 mL of HNO_3 25% (w/w) and a drop of silicone, to prevent foaming, were added. Afterwards, a glass device (described in 2.1) was placed in the solution and ozone was passed through for 10 minutes. The procedure was carried out at room temperature. After the ozonation process, the obtained suspension was centrifugated for 2 min at 3000 rpm. The centrifugation step can be avoided, but a clear supernatant was consider better than the use of the obtained slurry since the homogeneity of the sample is important for reproducible results when it is injected into the graphite furnace. The supernatant was used for the analytical determinations by ET-AAS. All the sample preparation procedures were carried out in a fume hood. Reagent blanks were measured alongside the samples.

3. Results and Discussion

3.1. Optimization

The influence of two critical variables (concentration of nitric acid and ozonation time) was studied using a three-level central composite design [20].

Table 2 summarizes the experimental design conditions.

Table 2. Central composite experimental design: two variables – three levels

Experiment	Concentration of HNO_3 (% w/w)	Ozonation time (min)	% R (Cd) (mean %; n=3)	% R (Pb) (mean %; n=3)
1	15	5	70.5	76.1
2	50	5	102.7	85.3
3	25	10	**100.2**	**93.2**
4	15	25	89.1	83.2
5	25	25	105.6	83.6

The optimal conditions for the sample preparation procedure were selected evaluating the recovery percentage estimated as R (%) = obtained concentration (mg kg^{-1}) x 100 / certified/informed concentration (mg kg^{-1}) for each experiment. The most simple and rapid experiment with an R (%) statistically equal to 100% (for both elements) was selected as the optimal for the subsequent validation. The results (dry-mass basis) for each experiment are showed in Table 2.

When 5 minutes of time interval is used and the acid concentration varies according to experiments 1 and 2, it can be observed that increasing the concentration of HNO_3 improves the recovery of both metals. For experiment 1 neither the concentration of ozone nor the concentration of acid is enough for quantitative extraction of the studied metals. For experiment 2, the recoveries are better probably due to the higher concentration of HNO_3 used. The best results were obtained in the conditions of the experiment 3, increasing the ozonation time to 10 minutes with a concentration of 25% of HNO_3. These results show that increasing the time of ozonation has influence on the process improving recoveries of both metals.

The variation of ozone concentration in water (in absence of other oxidant agents), was determined changing the time that the gas is bubbled in the solution and a linear relationship was observed up to 25 minutes. After this time, there was not more variation; this means that the maximum concentration was reached under the operative conditions of the equipment (saturation). This can be the cause of the results in experiments 4 and 5, compared with experiment 3, where no improvement was observed by increasing time or the acid concentration. The recoveries even decreased in these experiments.

Once the process was completed, the obtained suspension was centrifuged and the supernatant was used for the determination of Cd and Pb by direct calibration with aqueous standards. The entire process takes only 12 minutes.

This simple procedure does not require drastic conditions of sample preparation. It also uses an economical and easy to maintain equipment. The ozone generator costs USD 2500 in the region.

3.2. Figures of Merit

The validation was carried out using the experimental conditions described in the experiment 3.

For the evaluation of linearity, a blank and 3 standard solutions in the range 0.3 - 4 µg L^{-1} for Cd and 2-50 µg L^{-1} for Pb were measured (n =3) and the results were plotted as a function of the concentration. Linearity of the resulting curves was confirmed by visual inspection of the plot and analysis of residuals. Detection (LD, 3s) and quantification (LQ, 10s) limits were estimated by measuring the dispersion of the blank signal (n=10) and referring the measurements to the calibration curve. The LD/LQ presented in Table 3 corresponds to 0.002 mg kg^{-1}(Cd)/0.01 mg kg^{-1} (Pb) and 0.007 mg kg^{-1} (Cd)/ 0.03 mg kg^{-1} (Pb) respectively expressed on spinach leaves (dry- mass basis).

Analytical repeatability, expressed as relative standard deviation (RSD (%)), for the analysis of the CRM (n = 5) was 5.7% for Cd and 9.1% for Pb. The figures of merit are summarized in Table 3.

Table 3. Analytical figures of merit

	Result	
	Cd	Pb
Linearity	Up to 4.0 µg L^{-1} (r^2= 0.998)	Up to 50.0 µg L^{-1} (r^2= 0.998)
LD (3σ; n= 10)*	0.09 µg L^{-1}	0.6 µ L^{-1}
LQ (10 σ; n= 10)*	0.3 µg L^{-1}	1.9 µ L^{-1}
Precision (RSD %; n=5)	5.7	9.1

* Aqueous solution

As it is presented in Table 4, all the experimental t - values were below the theoretical t (0.05, 4) 2.78. Thus it may be concluded that the concentrations obtained with the proposed method do not differ significantly from the certified value/informed value, and the trueness of the method is ensured for these samples.

Table 4. Metal contents found in the certified reference material (NIST 1570 a) and comparison with reference value by Student´s *t*-test

	Certified value	Proposed Method (mean ± s; n=5)	% R	*t*-experimental
Cd (mg kg^{-1})	2.876 ± 0.058	2.82 ± 0.16	97.6	-0.787
Pb (mg kg^{-1})	0.2 *	0.197 ± 0.018	98.3	-0.415

*NIST informed value; s: standard deviation; t (0.05, 4) = 2.78 [21].

It is worth mentioning that for Cd the certified value is 10 times higher than the allowed by the regulations, so it is expected that the dilution factor will be different for other samples. According to the information provided by NIST, the CRM consists of spinach leaves. Although it was purchased with the objective of evaluating the reliability of analytical methods, to be a natural material contains an impressive amount of Cd.

This method was further applied to determine Cd and Pb in artichoke leaves. The MERCOSUR regulation allows a maximum of 0.4 mg kg^{-1} for Cd and 0.6 mg kg^{-1} for vegetables to prepare infusions (teas). The results were: (0,174 ± 0,022) mg kg^{-1} for Cd and (0,290 ± 0,030) mg kg^{-1} for Pb expressed as (mean ± s), n=3, in dry-mass basis. These values were in good agreement compared with those obtained performing microwave digestion (0.185 and 0.319 mg kg^{-1} respectively).

These results show that artichoke leaves comply with the regulation and therefore can be used to prepare infusions without health risks.

These are very promising results and encourage further investigation on the possible mechanisms involved. Probably a synergetic effect due to the use of an oxidizing acid in combination with an oxidizing agent such as ozone, and a very efficient agitation due to the bubbling of the gas are responsible for this success.

In published studies, the use of ozone is combined with other sources of energy such as ultrasound or UV, in this work the method only uses ozone, and the entire process was carried out at room temperature.

This method can be postulated as an alternative for sample preparation since the figures of merit are adequate to monitor compliance with the international regulations and food control, for both contaminants in spinach and artichoke leaves.

4. Conclusions

A novel method for the sample preparation of spinach and artichoke leaves for the determination of Cd and Pb using ET-AAS was optimized and validated.

This procedure proved to be adequate for the purpose of control of these inorganic contaminants in spinach leaves according to the regional regulations.

The method is simple, fast and in good agreement with Green Chemistry, with the novelty of the use of an ozonation procedure for sample preparation.

Acknowledgement

Agencia Nacional de Investigación e Innovación – ANII (Grant: POS_NAC_2013_1_11407), PEDECIBA-Química and Comisión Sectorial de Investigación Científica (CSIC).

Competing Interests

The authors have no competing interests.

References

[1] Technical Regulation “Reglamento técnico MERCOSUR” (DEROGACIÓN DE LAS RES GMC Nº 102/94 y Nº 35/96) MERCOSUR/GMC/RES. Nº 12/11 Available: http://www.puntofocal.gov.ar/doc/r_gmc_12-11.pdf [Accessed, May 18, 2015].

[2] Nobrega, J. A., Trevizan, L.C., Araujo, G.C.L. & Nogueira, A.R.A. “Focused-microwave-assisted strategies for sample preparation. A review”. *Spectrochim. Acta Part B*, 57, 1855-1876. 2002.

[3] Bendicho, C., de la Calle, I., Pena, F., Costas, M., Cabaleiro, N. & Lavilla, I. “Ultrasound-assisted pretreatment of solid samples in the context of green analytical chemistry”. *TrAC*. 31, 50-60. 2012

[4] Capelo, J. L., Pedro, H. A. & Mota A. M. “Ozone treatment for mercury determination in white wines.” *Talanta*, 61, 485-491. 2003.

[5] Capelo, J. L., Maduro, C. & Mota, A. M. “Advanced oxidation process for degradation of organomercurials: determination of inorganic and total mercury in urine by FI-CV-AAS”. *J.Anal. At. Spectrom*. 19, 414-416. 2003.

[6] Kim, M. J. & Nriagu, J. “Oxidation of arsenite in groundwater using ozone and oxygen”. *Sci. Total Environ*. 247, 71-79. 2000.

[7] Audenert, W.T.M., Vandierendonck, D., Van Hulle, S.W.H. & Nopens, I. “Comparision of ozone and HO. Induced conversion of effluent organic matter (EfOM) using ozonation and UV/H2O2 treatment”. *Water Res*. 47, 2387-2398. 2013.

[8] Khuntia, S., Majumder, S.K. & Ghosh, P. “Oxidation of As (III) to As (V) using ozone microbubbles”. *Chemosphere*, 97, 120-124. 2014.

[9] Domenjoud, B., Tatari, C., Esplugas, S. & Baig, S. “Ozone-Based Processes Applied to Municipal Secondary Effluents”. *Ozone-Sci.Eng*. 33, 243–249. 2011

[10] Sponza, D. T. & Oztekin, R. “Effect of Ultrasonic Irradiation on the Treatment of Poly-Aromatic Substances (PAHs) from a Petrochemical Industry Wastewater”. *Ozone-Sci.Eng*. 33, 194-210. 2011.

[11] Whangchai, K., Uthaibutra, J., Phiyanalinmat, S., Pengphol, S. & Nomura, N. “Effect of ozone treatment on the reduction of Chlorpyrifos residues in fresh lychee fruits.” *Ozone-Sci. Eng*. 33, 232-235. 2011.

[12] Quero-Pastor, M., Valenzuela, A., Quiroga, J. M. & Acevedo, A. “Degradation of drugs in water with advanced oxidation processes and ozone”. *J. Environ. Manage*. 137, 197-203. 2014.

[13] Matusiewicz, H. & Mroczkowska, M. “Hydride generation from slurry samples after ultrasonication and ozonation for direct determination of trace amounts of As(III) and total inorganic arsenic by their in situ trapping followed by graphite furnace atomic absorption spectrometry”. *J. Anal. At. Spectrom*. 18, 751-761. 2003.

[14] Capelo, J.L., Maduro, C. & Mota, A.M. “Evaluation of focused ultrasound and ozonolysis as sample treatment for direct determination of mercury by FI-CV-AAS. Optimization of parameters by full factorial design”. *Ultrason. Sonochem*. 13, 98-106. 2006.

[15] Pistón, M., Machado, I., Branco, C.S, Cesio, V., Heinzen, H., Ribeiro, D., Fernandes, E., Chisté, R.C. & Freitas, M. “Infusion, decoction and hydroalcoholic extracts of leaves from artichoke (*Cynara cardunculus* L. subsp. cardunculus) are effective scavengers of physiologically relevant ROS and RNS”. *Food Res. Int*., 64, 150-156. 2014.

[16] Arpadjan, S., Celik, G., Taskesen, S. & Gücer, S. “Arsenic, cadmium and lead in medicinal herbs and their fractionation.” *Food and Chemical Toxicology*, 46, 2871-2875. 2008.

[17] National Institute of Standards and Technology (NIST) Certificate of Analysis Standard Reference Material® 1570a. Trace elements in spinach leaves. Available: https://www-s.nist.gov/srmors/view_cert.cfm?srm=1570a [Accessed May 18, 2015].

[18] APHA; AWWA; WPCF. Standard methods for the examination of water and wastewater. Washington: American Public Health Association. 2005.

[19] Santos, J., Oliva-Teles, M.T., Delerue-Matos, C., Oliveira M.B.P.P. “Multi-elemental analysis of ready-to-eat ‘‘baby leaf’’ vegetables using microwave digestion and high-resolution continuum source atomic absorption spectrometry”. *Food Chemisty,* 151, 311-316. 2014.

[20] Massart, D. L, Vandeginste, B.G.M., Buydens, L.M.C., de Jong, S., Lewi, P.J. & Smeyers-Verbeke, J. *Handbook of Chemometrics and Qualimetrics: Part A*, Amsterdam: Elsevier Science, 1997, Chapter 24.

[21] Miller, J. N. & Miller, J.C. (1993). *Estadística para Química Analítica*. (2nd ed.), Addison-Wesley Iberoamerican S.A. Wilmington, 1993.