Ultrasound-assisted pretreatment for multielement determination in maize seed samples by microwave plasma atomic emission spectrometry (MPAES)

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ABSTRACT

In this work, a rapid and simple ultrasound-assisted pretreatment for maize samples is proposed. Also, the performance of microwave plasma atomic emission spectrometry (MPAES) for the analysis of Ag, Al, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, Pb, Tl, V and Zn in maize seeds, was evaluated. Instrumental parameters optimization, as well as validation and application of the MPAES are presented. The obtained limits of detection were ranged between 0.7 µg g^{-1} (for Mo and Fe) and 4.3 µg g^{-1} (for Ca). Validation assays were carried out by standard addition and internal standard, showing good recovery performances, over 96% in all cases. The developed method was applied to trace elements determination of Argentinean maize samples, improving the productivity performance due the reducing costs — by the use of atmospheric nitrogen as plasma source for MPAES — and time analysis, which could be trustworthy for routine analysis, quality control and traceability of maize.

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1. Introduction

The agricultural activity is one of the fundamental pillars upon which the Argentinean economy is built [1–2]. Due to this contribution, the evaluation of certain compounds that affect nutritional quality in crops becomes relevant. The multielemental profile constitutes an important crop quality factor in relation to physiological stress, as well as for human health, due to its significance in biological systems and in food product quality [3].

Absorption and accumulation of heavy metals and trace elements in plants depends on their transport from soil solution to roots, from stems to leaves, as well as from leaves to the plant's storage tissues (as seeds, grains or fruits) [4–7]. From the point of view of plant nutrition, at least 14 mineral elements are required for full physiological activity. According to the element, the range of concentration varies from >10,000 µg g^{-1} (e.g. Ca, N, K), to 0.001 µg g^{-1} (e.g. Mo, Ni). Each element is required within an optimum range, which if it is exceeded, will induce deficiency or toxicity symptoms with consequences for plant health and survival, which also will impact in the product quality [6].

Several studies on the effect of metals on some crops have been carried out, focusing on contaminated or fertilized soils, where the influence of those metals is observed in the plant growing. In this way, a few studies of elemental determination on maize seeds in contaminated soils have been reported [8–10]. Since maize production is relevant in Argentina, where it is used as food source, it is important to know elemental profile in seeds, because there is not any previous reference on the matter.

Elementomics involve elemental profile studies in living organisms, requiring the development of different analytical methodologies. Thus, it is necessary to have modern, sensitive and selective techniques due to the low levels concentrations of elements in samples, as well as the complex matrices where they could be found. Thereby, microwave plasma atomic emission spectroscopy (MPAES) is one of the most versatile analytical methods available today. The main advantages of MPAES are related to the simultaneous multielement detection, high sensitivity, wide range of linear response and low noise level compared to other methods, allowing the detection of a large number of elements ranging from alkali- metals, rare earths and transition elements or even some non-metals [7,11–16]. On the other hand, the use of atmospheric nitrogen instead argon as plasma source, reduces significantly the cost of analysis.

In the elemental analysis of plants, sample preparation is a critical stage in the analytical procedure. The organic matrices represent a

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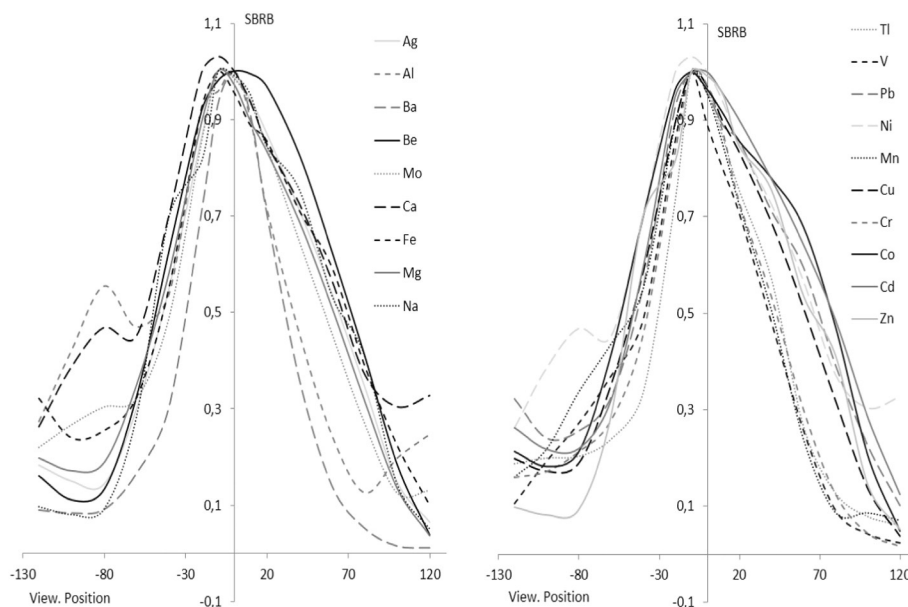
Fig. 1. Viewing position in MPAES as function of SBRB (signal to back ground ratio) to obtain the optimal analytical signal for the analytes studied.

challenge for conventional introduction systems in the emission spectrometer, which generate several troubles such as plasma instability, organic matter remnant and interferences among others. The complexity of the maize organic matrix, resulting of its high starch, fat and fiber contents, requires a thorough mineralization before multielemental determination increasing the analysis time for a great number of samples, but also the possibility of contamination since many steps are added.

As an alternative for sample preparation by conventional microwave system, a fast and simple ultrasound-assisted sample mineralization by heating in acid media (HNO_3) is proposed. The new analytical sample pretreatment have been employed for multielemental determination in Argentine maize crops, by microwave plasma atomic emission spectrometry (MPAES), diminishing analysis time and improving the performance and productivity.

2. Experimental

2.1. Instrumentation

Elemental determination was performed using an Agilent MP-AES MP 4100 (Santa Clara, USA) which includes an inert One Neb nebulizer, a double-pass glass cyclonic spray chamber and a SPS3 auto-sampler system. A Czerny-Turner monochromator with a charge-coupled device (CCD) array detector was used in this study. MP AES 4100 operates with on line nitrogen generator (Agilent 4107). The plasma gas flow was fixed at 20 L min^{-1} and the auxiliary gas flow was fixed at 1.5 L min^{-1}. For the analyses of all elements common settings have been used: the uptake time 13 s, the plasma stabilization time with sample aspiration 15 s, read time 3 s (read in triplicates), and wash time 20 s. The mode of automatic background correction has been
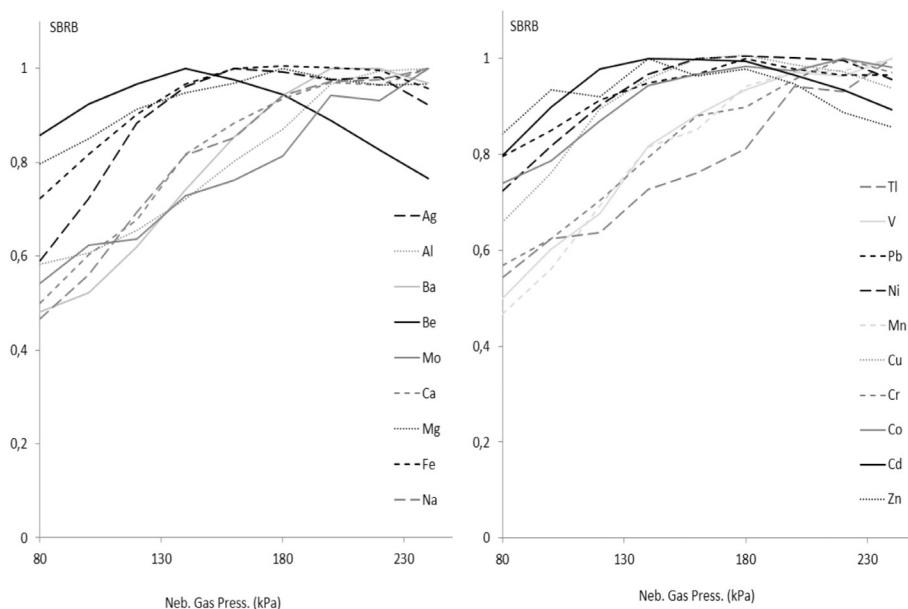

Fig. 2. Changes of the analytical signal with increasing of MPAES nebulizer pressure as function of SBRB (signal to background ratio) for the analytes studied.

used (off-peak left, off-peak right or off-peak left + right). The operating parameters such as viewing position and nebulizer pressure were optimized for each element. The microwave mineralization was carried out using an Anton Paar MW 3000 microwave system (Graz, Austria). An Ultrasound (Testlab) with a frequency of 40 kHz, a power of 160 W and 30 samples at time, was used.

2.2. Reagents

Ultra-pure deionized water (resistivity of 18.2 mΩ) was produced by a Millipore ultra-purifier for all solutions (Darmstadt, Germany). The glass material used throughout the study was cleaned with 10% nitric acid for 24 h before use and washed with deionized water. To perform the mineralization of samples, concentrated HNO_3 (Merck, Darmstadt, Germany) was obtained by distillation into a Berghof suboiling distiller system (Eningen, Germany). For calibration step, multielement standards were used in a matrix of 5% HNO_3 (100 µg mL^{-1}, Science Plasma Cal). For recovery, Indium Standard (Sigma-Aldrich TraceCERT®, 1000 mg L^{-1} In in nitric acid) was used.

2.3. Sampling

Samples were collected from cultivated areas of the Argentina's central region (including La Pampa, Córdoba and Buenos Aires provinces). Sampling was conducted during harvest time, from September (2014) until March (2015): a total of 10 samples were collected and analyzed.

2.4. Analytical procedure for sample preparation

Maize seeds were milled and stored in plastic bags until analysis. Before mineralization, the milled seeds were dried in an oven at 60 °C for 24 h. For ultrasound method, 0.5 g of dry samples were accurately weighted and transferred to tubes with caps. Samples were added with 5 mL HNO_3 and placed into a thermostatted water bath at 100 °C for 30 min; then, acid samples were placed into ultrasound equipment for the same period of time, cooled at room temperature and diluted to 30 mL with deionized water. For comparative studies microwave mineralization was carried out as follow: 0.5 g of dry samples were accurately weighted, transferred to a hermetically sealed 100 mL PTFE tube and added with 8 mL HNO_3; tubes were placed in the Anton Paar microwave system and a two-step program (15 min up to 190 °C and up to 1200 W and kept by 15 min at 190 °C, up to 1200 W) was used. After that, all samples were transferred to a 30 mL volumetric tube and diluted with deionized water to mark.

Table 1
Optimized parameters for metals determination by MPAES.

Element	Wavelength (nm)	Viewing position	Nebulizer pressure (kPa)
Ag	328.068	0	200
Al	396.152	0	220
Ba	455.403	0	200
Be	234.861	−10	120
Ca	422.673	0	100
Cd	228.802	−10	140
Co	340.512	−10	220
Cr	324.433	0	240
Cu	324.754	0	180
Fe	259.94	−10	200
Mg	383.829	−10	200
Mn	403.076	0	240
Mo	386.41	−10	240
Na	589.592	0	240
Ni	352.454	−10	160
Pb	283.305	40	180
Tl	535.046	0	240
V	437.923	−10	240
Zn	481.053	−10	160

Table 2
The *t* test performed on by microwave (MW) and ultrasound (US) systems on five elements.

Analyte	MW*	US*	*t* cal
Cu	31.4	34.2	0.63
Fe	19.5	21.0	0.43
Mg	621	752	0.77
Mn	7.2	7.6	0.52
Zn	17.5	20.1	2.08

* Mean values µg g^{-1} (n = 5).

2.5. Data analysis

All calculations carried out in this work were done in MATLAB 2014b, environment. Environment based on the well-known routines (available in www.iquironicet.gov.ar/descargas/univar.rar).

3. Results and discussion

3.1. Instrument optimization

3.1.1. The viewing position
The microwave plasma is vertically-oriented to improved matrix handling, but it has axial viewing for optimum sensitivity and best detection limits. To optimize instrumental conditions, initially the best viewing position in the instrument was assessed. Thus, a plasma viewing position was optimized for each element wavelength to reach the best analytical performance. Fig. 1 shows the obtained results for the optical calibration using standard solutions.

3.1.2. Nebulizer gas pressure
To obtain the highest analytical signal, the nebulizer pressure for each element was optimized. The changes of analytical signal as function of gas pressure in nebulizer are shown in Fig. 2. In almost cases, all elements had the best signal close to 240 kPa. Table 1 resumes the optimized parameters for each element.

3.2. Pretreatment of samples

As maize possesses a complex organic matrix, as consequence of starch, fat and fiber, it requires a complete mineralization. Accordingly, sample treatments were accomplished to reach the best result that enables introduction to MPAES without trouble; for this reason, an

Table 3
Analytical performance obtained for all elements by ultrasound mineralization and MPAES.

Element	LOD (µg g^{-1})	LOQ (µg g^{-1})	RSD (%)	R^2
Ag	1.3	3.9	0.8	0.9999
Al	0.9	2.7	0.6	0.9999
Ba	1.7	5.1	0.1	0.9997
Be	1.1	3.3	0.3	0.9996
Ca	4.3	12.9	1.8	0.9964
Cd	1.1	3.3	1.0	0.9998
Co	0.9	2.7	5.8	0.9993
Cr	0.8	2.4	0.8	0.9999
Cu	1.4	4.2	0.9	0.9999
Fe	0.7	2.1	3.0	0.9988
Mg	3.6	10.8	2.6	0.9991
Mn	1.2	3.6	0.5	0.9999
Mo	0.7	2.1	0.1	0.9999
Na	2.9	8.7	4.4	0.9999
Ni	1.3	3.8	0.7	0.9999
Pb	1.7	5.2	4.6	0.9992
Tl	1.0	3.0	0.6	0.9999
V	1.1	3.3	0.8	0.9999
Zn	1.7	5.1	1.7	0.9998

Table 4
Results of elemental analysis performed by MPAES on 10 maize seed samples. Concentrations expressed in μg g^{-1} (dry weight).

Analyte	1	2	3	4	5	6	7	8	9	10
Al	ND	<LOQ	2.9 ± 1.5	ND	ND	4.8 ± 2.4	<LOQ	<LOQ	<LOQ	<LOQ
Ba	ND	<LOQ	<LOQ	ND	<LOQ	<LOQ	<LOQ	<LOQ	ND	ND
Ca	<LOQ	<LOQ	<LOQ	<LOQ	5.61 ± 2.62	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Cu	<LOQ	18.2 ± 1.3	29.0 ± 2.0	<LOQ	<LOQ	52.8 ± 18.5	32.0 ± 19.4	20.8 ± 25.2	28.9 ± 1.3	36.4 ± 1.0
Fe	16.4 ± 4.6	14.0 ± 0.9	17.0 ± 9.7	22.0 ± 7.6	19.2 ± 6.0	84.9 ± 10.7	18.7 ± 8.5	15.9 ± 7.7	16.7 ± 6.7	18.9 ± 1.0
Mg	888 ± 67	572 ± 51	625 ± 50	993 ± 99	908 ± 56	813 ± 39	458 ± 18	510 ± 22	740 ± 71	437 ± 29
Mn	5.7 ± 0.6	7.0 ± 0.7	6.9 ± 1.3	5.7 ± 1.9	5.5 ± 0.7	15.4 ± 6.0	17.4 ± 7.9	6.8 ± 0.2	11.4 ± 0.7	6.7 ± 1.0
Ni	4.8 ± 0.4	3.8 ± 0.4	4.9 ± 0.5	5.0 ± 0.8	<LOQ	3.8 ± 1.0	3.8 ± 1.1	4.3 ± 0.2	3.8 ± 0.3	4.9 ± 0.1
Pb	19.7 ± 4.1	3.8 ± 2.6	5.3 ± 0.8	<LOQ	<LOQ	6.0 ± 1.9	7.5 ± 2.9	5.6 ± 1.6	6.8 ± 1.4	5.4 ± 1.1
Zn	ND	13.1 ± 1.7	14.2 ± 1.0	ND	ND	25.9 ± 7.9	16.4 ± 8.3	13.2 ± 0.3	18.1 ± 1.1	22.5 ± 1.6

ultrasonic-assisted treatment was developed. The proposed method was statistically compared with a conventional microwave mineralization using a t test (α = 0.05, n = 10), results are shown in Table 2. It can be observed that there are not significant differences between treatments (critical t value = 2.306) demonstrating that the ultrasound-assisted mineralization method can be proposed as an interesting and non expensive option for the pretreatment of maize samples. On the other hand, microwave system has a 16-position rotor allowing only few samples, whereas the proposed ultrasound-assisted treatment enables higher number of samples in a short period of time [17–18]. Therefore, in spite of the fact that microwave oven is secure for user, the ultrasound-assisted method has more advantages. As can see in Table 2, only 5 elements were studied, because these elements were the unique found in all analyzed samples which indicate its heterogeneity in terms of composition.

3.3. Analytical performance

Table 3 displays the figures of merit for trace element determination in maize samples after ultrasound-assisted treatment. The recommended ultrasound assisted procedure reaches detection limits (LOD) between 0.7 μg g^{-1} for Mo and Fe, and 4.3 μg g^{-1} for Ca; with adequate precision as relative standard deviation (RSD) from 0.1% for Ba to 4.6% for Pb; r^2 coefficient major than 0.999 were obtained from each element calibration curve.

3.4. Analytical application: Elemental profile in maize seed samples

Table 4 displays the results of multielement determination by MPAES carried out in ten seed maize samples collected in Argentine after ultrasound-assisted mineralization. The concentrations of Ag, Be, Cd, Co, Mo, Tl and V were under LOD, while Cr and Na were under LOQ. The occurrence of differences on elemental composition of samples, which could be observed for several elements i.e. Al, Ca, Cu, may be attributed to different sample origins, indicating a possible effect of the geographical origin on the results. In the case of toxic elements, maize showed high amounts of Pb, but under the maximum limit recommended by WHO (10 μg g^{-1}) except to sample one [19]: the presence of Pb in maize seeds can be due to use of some fertilizers which can add this element to soil [20–21].

Table 5
Recovery degree obtained for several elements (n = 10).

Analyte	Recovery (%)	RSD (%)
Cu	99.5	3.3
Fe	108.7	9.7
Mg	109.4	9.9
Mn	104.3	4.5
Mo	93.1	9.6
Na	104.7	5.6

3.5. Analytical validation

To assess the presence of systematic error during the ultrasound-assisted mineralization step, an analytical validation was performed for each analyte (Cu, Fe, Mg, Mn, Mo and Na), for the same matrix sample, in the same range of concentrations [22]. The individual recoveries for every element were ranged from 93–109% and are showed in Table 5.

3.6. Recovery assay

To evaluate the recovery of the proposed pretreatment method, the addition of 100 μg L^{-1} Indium as internal standard to all samples was performed. The obtained performance for the recovery of In was 94 ± 9% (n = 10).

4. Conclusions

Maize samples were suitably mineralized by ultrasonic-assisted treatment, enabling their introduction to the MPAES. This sample preparation could be an attractive alternative for routine analysis, considering mainly the multielement analysis capabilities of MPAES for complex matrices with high carbon content and the benefits of using air for nitrogen plasma generation, which reducing significantly the running cost. Thus, the proposed method is fast, simple, precise, accurate and less expensive than the microwave system. Also, the concentrations of 10 analytes determined in Argentinean maize seeds (including major, minor and toxic elements) were reported for this crop. Pb concentrations in maize samples were lower than the maximum permissible by the WHO, except in one sample. Results from this study emphasized the importance of the multielement determination of trace elements in maize samples to carry out traceability and/or origin denomination studies and also to assess their quality. However, more studies must be carried out to include maize samples from other regions around the world.

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