Determination of elements in cereals, pseudocereals, and legumes by microwave plasma-atomic emission spectrometry

Jessica L. Braden [1], Emily F. Klarquist [*,1], Julianne A. Kellogg

Department of Crop and Soil Sciences, Washington State University, Pullman, WA, USA

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ABSTRACT

A novel method for multi-element analysis in cereals, pseudocereals, and legumes was developed for principal (calcium, magnesium, potassium, and phosphorus) and trace (manganese, zinc, iron, copper, and aluminum) element determination using a microwave plasma-atomic emission spectrometer (MP-AES). The method was validated using certified reference analyte values from durum wheat (DUWF-1), corn bran (BRAN-1), quinoa (KINO-1), rice (SRM 1568b), and soy (SRM 3234). Spike recoveries were assessed using field-grown crops that represent staple and minor crops with variable matrix compositions. A closed-vessel microwave-assisted digestion method consisting of 12 mL of deionized water, 2 mL of HNO3, and 2 mL of H2O2 was efficient for the mineralization of all crops. Acceptable measurement agreement was achieved between certified and determined values for all reference materials with recovery ranges from 89 to 120 percent. Plant breeders can use the method to develop and screen crops for improved nutrient density.

1. Introduction

In response to the persistent rise in hunger and malnutrition globally, priorities in the agricultural sector have shifted from research focusing solely on calorie-rich staple foods to including research and development of 'nutrient-rich foods' (Poole et al., 2021). The integration of nutrition-related traits in cultivar development programs for human health and nutrition security has gained momentum over the last decade due to high-throughput phenotyping methodologies, like X-ray fluorescence spectroscopy (XRF) (Govindan et al., 2021; Poole et al., 2021). XRF has been critical in mainstreaming biofortification efforts across plant breeding programs to develop cultivars with higher concentrations of zinc and iron (Guild, 2017). However, its application as a quantitative method is limited to only a few minerals, as it is prone to matrix effects that lead to spectral interferences and higher detection limits compared to other analytical methods (Guild et al., 2017; Paltridge et al., 2012). This limitation is especially pronounced for lighter elements such as potassium and magnesium, for which no calibration using whole seed samples has been reported. Consequently, analytical detection methods such as inductively coupled plasma-optical emission spectrometry (ICP-OES) and -mass spectrometry (ICP-MS) are required in research where detailed characterizations of the micronutrient composition in crops are required.

While ICP-based methods have been used in biofortification research (Guild, 2017), their application remains limited due to the extensive sample preparation required for analysis and the high costs associated with instrument acquisition and consumables (i.e., argon gas). Some of these barriers may be addressed using a microwave plasma-atomic emission spectrometer (MP-AES), which can provide adequate sensitivity for characterizing elemental concentrations at lower instrument and operating costs compared to ICP-based methods (Balaram, 2020; Fontoura et al., 2022). Due to the sequential elemental analysis used by the MP-AES, it is best suited for plant breeding programs where cost, and not time, is the primary limiting factor or when only a few elements are targeted for selection. When combined with XRF, the MP-AES enables the establishment of a comprehensive in-house biofortification program at a lower cost.

The validated MP-AES methods for crops have been limited in

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application to a single crop species and often utilize a low-tech, time-consuming digestion method (Bolaños et al., 2019; Heredia et al., 2016; Karlsson et al., 2015; Oliva et al., 2019; Ozbek et al., 2019). While a multi-crop MP-AES method would improve its usefulness in plant breeding, validating such a method can be challenging due to significant variations in composition across species coupled with the limited availability of certified reference materials (CRMs). To validate a method, researchers often rely on multiple CRMs to capture an extensive range of the analytes of interest (Otaka et al., 2014; São Bernardo Carvalho et al., 2020) or perform single analyte spike recoveries for assessing matrix effects and method accuracy (Guerrero Esperanza et al., 2017; Heredia et al., 2016; Nascimento et al., 2014). Spike recoveries from several representative matrices, in combination with CRMs, provide a robust validation strategy for a multi-crop MP-AES method (Sullivan & Carpenter, 1993).

To further increase the utility of the MP-AES in plant breeding, a multi-crop method using microwave-assisted acid digestion would increase the cost-effectiveness of micronutrient analysis for cultivar development by reducing the time for sample preparation and the cost of reagents. Microwave-assisted acid digestion is a closed-vessel digestion method that allows for faster (<60 min) and more complete sample digestions compared to open-vessel methods (Dolan & Capar, 2002). Due to the high temperatures reached in the pressurized system, diluted nitric acid can be used while still achieving desirable amounts of residual carbon content (RCC) and lower amounts of residual acid in sample digests (Adamczyk-Szabela et al., 2017; Lee et al., 2022; Lemos et al., 2019). When nitric acid is coupled with a small addition of hydrogen peroxide in this system, the oxidative potential can maintain RCC retention without increasing residual acidity (Araújo et al., 2002). These factors contribute to better analyte recoveries when compared to higher concentrated acid methods (Araújo et al., 2002; Barbosa et al., 2015), allowing for environmentally safer and lower-cost digestion methods suitable for multiple crop species with complex matrices.

The objective of this study was to develop a methodology to measure principal elements (Ca, Mg, K, and P) and trace elements (Mn, Zn, Fe, Cu, and Al) using the MP-AES across multiple crop matrices. The elements were selected for their nutritional significance to human health (Ca, Mg, K, P, Mn, Zn, Fe, and Cu) or used to indicate soil contamination in the sample (Al). Multiple matrices were used to demonstrate applicability for plant breeding programs working with several crop species. The method was validated using multiple certified reference materials consisting of durum wheat (DUWF-1), corn bran (BRAN-1), quinoa (KINO-1), rice (SRM 1568b), and soy (SRM 3234). Method accuracy was examined using analyte spike recoveries across several crop matrices that included amaranth (*Amaranthus cruentus* L.), barley (*Hordeum vulgare* L.), fava (*Vicia faba* L.), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.), maize (*Zea mays* L.), and lupin (*Lupinus mutabilis* L.).

2. Hypothesis

A MP-AES multi-element determination method can be validated for cereals, pseudocereals, and legumes.

3. Material and methods

3.1. Reagents and materials

Deionized water (DIW, 18.2 MΩ) from the Diamond Nanopure (Barnstead Lab Water Products, Lake Balboa, CA, USA) reverse osmosis filtration system was used in the analysis of all samples and preparation of reagents. ACS Grade HNO_3 (68–70 %, VWR, Radnor, PA, USA) and ACS Grade H_2O_2 (30 %, Avantor, Radnor, PA, USA) were used for all blanks and sample preparations. Certified 1000 ppm VeriSpec™ stock solutions (Ricca Chemical, Arlington, TX, USA) were used to make single-element calibration solutions, matrix spikes, and check solutions. The ionization buffer was prepared by dissolving the appropriate

amount of $CsNO_3$ salt (99.8 %, Alfa Aesar, Haverhill, MA, USA) to make a 0.25 % Cs in HNO_3 (4 %) solution. All reagents were stored at room temperature. Certified standards DUWF-1 (durum wheat), BRAN-1 (corn bran), and KINO-1 (quinoa) obtained from the National Research Council Canada (NRC, Ottawa, Ontario, Canada); and SRM 3234 (soy) and SRM 1568b (rice) from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) were used for method validation. All CRMs were stored at room temperature and in closed original packaging with indirect sunlight until analysis.

3.2. Instrumental

An Agilent MP-AES 4200 (Agilent Technologies, Santa Clara, CA, USA) equipped with a double pass glass cyclonic spray chamber, One-Neb V2 Nebulizer, and an SPS-3 autosampler (Agilent Technologies, Santa Clara, CA, USA) was used in this study. A Genius 3055 nitrogen generator (Peak Scientific, Inchinnan, Scotland, UK) extracted atmospheric nitrogen gas to sustain the plasma flame in the MP-AES. The analytical cycle consisted of 30s rinsing with 4 % nitric acid followed by 15 s of sample uptake and 15 s of stabilization before 3 s read time for all elements. The pump speed during analysis was kept at 15 rpm. The waste pump tubing (Agilent Technologies, Santa Clara, CA, USA) was blue/blue at 1.65 mm inside diameter (ID). The sample pump tubing was black/black at 0.76 mm ID, and the ionization buffer tubing was orange/green at 0.38 mm ID. The sample tubing and ionization buffer tubing were connected by a Y-fitting (Timberline Instruments, Boulder, CO, USA) positioned just before the sample reached the nebulizer.

3.3. Sample collection

A detailed outline of planting, sample collection, and sample preparation is described by Kellogg (2022). In brief, sampling activities occurred in the Cañar Province of Ecuador in November and December of 2020. Sample collection methods were adapted from Stangoulis and Sison (2008) to minimize sample contamination. Twelve primary samples (200 g) were collected from bulk storage and combined to create a 2400 g composite sample of each crop following the recommendations by Greenfield and Southgate (2003). The composite sample was mixed prior to collecting a final 300 g sample. The samples were stored in clear plastic bags until being mailed to Washington State University (Pullman, WA, USA) with appropriate phytosanitary certificates for each crop.

3.4. Sample preparation

Crop samples (300 g) were rinsed three times with DIW and immediately dried at 70 °C for 2 h; seeds were stirred every 30 min during drying. Samples were representatively subsampled (30 g) in duplicate using a stainless-steel Gilson Universal Mini Splitter (Gilson Company Inc., Lewis Center, OH, USA). Duplicate analytical samples were milled using an IKA A 10 Basic Mill (IKA Works Inc., Wilmington, NC, USA), as recommended by Stangoulis and Sison (2008). After milling, samples were weighed to collect fresh weight values. The processed analytical samples were stored in paper coin envelopes on desiccator beads in sealed containers at room temperature until analysis. To determine element concentrations on a dry weight basis, a separate 1 g sample was dried at 90 °C for 2 h, then allowed to come to room temperature after 10 min in a desiccator before obtaining the oven-dried weight.

3.5. Procedure

For all samples, 250 mg (+/− 5 mg) of flour was added to a 75 mL PTFE digestion vessel containing 2 mL DIW. An additional 10 mL of DIW and 2 mL of HNO_3 were added to all samples. The loaded vessels were capped and vortexed to incorporate the flour into acid for 1 min, followed by an addition of 2 mL of H_2O_2. Samples were then pre-digested for 15 min with the caps removed. For the spiked experiment, 2 mL of

Table 1
Mars6 Xpress Microwave System heating program.

Heating Program		
Step 1	Ramp temp (C)	120
	Ramp time (min)	10
	Hold time (min)	2
	Power (W)	1800
Step 2	Ramp temp (C)	210
	Ramp time (min)	20
	Hold time (min)	15
	Power (W)	1800

Table 2
Operating conditions for element determination in crop samples in the proposed MP-AES method.

Element	Wavelength (nm)	Nebulizer Flow (L min^{-1})	Calibration Fit	Calibration Range (mg kg^{-1})
Ca	616.217	0.6	Linear	2–20
Mg	285.213	0.75	Rational	2–20
P	213.618	0.4	Linear	10–40
K	404.414	0.75	Rational	10–140
Fe	371.993	0.55	Linear	0.5–20
Zn	213.857	0.45	Linear	1–6
Mn	403.076	0.65	Linear	0.02–4
Cu	324.754	0.65	Linear	0.02–4
Al	396.152	0.8	Linear	0.05–4

stock solution of a single analyte was added in place of the 2 mL DIW before the sample was added to the vessel. Analyte spikes were added at 0.5× or 1× the sample concentration based on the approximate analytical concentration of the crop sample (Supplementary Table 1).

All digestions were carried out using the Mars6 Xpress Microwave System (CEM Corporation, Matthews, NC, USA) equipped with 40 PTFE vessel holders. Due to the high volume of water in sample extractions and the power needed for mineralization, the digestion run was limited to sets of 10 samples. The digestion program (Table 1) was modified from Barbosa et al. (2015) for a total digestion time of 47 min. Following recommendations by CEM Corporation, the power was set to 1800 W but did not exceed 1200 W. After cooling for at least 30 min, the samples were transferred to 50 mL centrifuge tubes and diluted to volume with DIW.

3.6. Optimization of MP-AES

The operational conditions of the MP-AES are summarized in Table 2. The signals from the MP-AES were evaluated with the original software, MP Expert (Agilent Technologies, Santa Clara, CA, USA). The viewing position was set to zero, and the nebulizer flow (L min^{-1}) rates were determined based on prior analyte recoveries during method development and entered manually. The calibration range for each analyte was determined by establishing the needed range for our sample concentrations, selecting the appropriate sensitivity of wavelengths, and setting the acceptable correlation coefficient at >0.99 for all analytes. A total of 5–6 standards were used for each analyte calibration. The calibration range for each element was (in mg kg^{-1}) Ca (2–20), Mg (2–20), P (10–40), K (10–140) Fe (0.5–10), Zn (1–6), Cu (0.02–4), and Al

(0.05–4). The calibration fit was rational for Mg and K and linear for Al, Ca, Cu, Fe, Mn, P and Zn. Automatic background correction and five technical replications were used for all analyses. Each analysis included one replicate of a blank standard, digestion-method blank, mid-calibration range standard and below calibration range standard.

3.7. Statistics

Seven replicates of each certified reference material were used to validate the method. The average analyte estimates were used to calculate estimated uncertainty and assess agreement with the certified value. The estimated uncertainty of an analyte ($u(\overline{x}_{analyte})$) for each CRM was established using the following equation from Sharpless and Duewer (2008):

$$u(\overline{x}_{analyte}) = \sqrt{\left(\frac{s_R^2}{x_{CRM}}\right)\bigg/ n + \left(\frac{U_{95}(x_{CRM})}{2}\right)^2}$$

where s_R is the estimated long-term reproducibility, determined from the RSD obtained from the MP-AES, and n is the number of replicates ($n = 7$) used in validation. The x_{CRM} is the certified value for the analyte with uncertainty $U_{95}(x_{CRM})$.

All analyte recoveries ($Recovery_{CRM}$, %) for CRMs were averaged across replicates and calculated as follows:

$$Recovery_{CRM}, \% = \frac{\overline{x}_{analyte}}{\overline{x}_{CRM}} x\ 100$$

where $\overline{x}_{analyte}$ is the average concentration ($n = 7$) of the reference material determined using the proposed method, and $\overline{x}_{CRM}$ is the certified value.

Three replicates of each matrix-spike were used to calculate recovery. Spike concentrations were calculated from the analytical portion of the sample and did not exceed 100 % of the analytical portion concentration. Matrix spike results are reported by percent recovery ($Recovery_{spike}$, %) using the following equation:

$$Recovery_{spike}, \% = \frac{(C_f - C_u)}{C_a} x\ 100$$

where C_f is the concentration of the fortified or spiked sample, C_u is the concentration of the unfortified sample, and C_a is the concentration added to the sample.

4. Results and discussion

4.1. Ionization buffer

During the method development phase, elemental interference led to an overestimation of calcium. When using atomic emission lines, the chance for elemental interference generally decreases. However, switching to the atomic emission line was ineffective due to many easily ionizable elements present in the sample. To correct this, a more easily ionizable element (e.g., cesium) that is not naturally occurring in the sample reduced the ionization of the elements of interest during analysis and lead to better elemental recoveries. At a concentration of 0.25 % Cs, the ionization buffer was sufficient in improving the recovery of calcium

Table 3
Analytical figures of merit of the proposed MP-AES method. LOD = limit of detection, LOQ = limit of quantification, RSD = relative standard deviation.

Parameter	Ca	Mg	P	K	Fe	Zn	Mn	Cu	Al
r^2	0.9995	0.9999	0.9999	0.9991	0.9996	0.9990	0.9979	0.9996	0.9999
LOD (µg L^{-1})	7.8	2.3	169.6	179.6	2.8	3.6	1.2	4.3	0.7
LOQ (µg L^{-1})	26.1	7.8	565.2	598.7	9.2	12.0	4.2	14.2	2.4
RSD (%)	3.0–10.3	2.0–3.6	1.6–4.1	3.0–6.8	3.0–8.6	2.0–9.6	4.0–6.3	4.0–17.1	5.6

Table 4

Comparison of determined values (*n* = 7) and certified values for reference materials. ND = not determined, NF = none found, *estimated uncertainty did not overlap with certified reference values.

	Experimental Method		Certificate of Analysis	
	Determined value (mg kg⁻¹)	Estimated uncertainty	Certified value (mg kg⁻¹)	Expanded uncertainty
Calcium				
DUWF-1	298	26	278	26
BRAN-1	472	50	420	38
KINO-1	728	100	720	100
SRM 3234	3248	89	3191	56
SRM 1568b	123	5.34	118.4	3.1
Magnesium				
DUWF-1	1034	80	1070	80
BRAN-1	797	62	818	59
KINO-1	1906	300	1970	300
SRM 3234	3361	84	3487	60
SRM 1568b	498	10.93*	559	10
Potassium				
DUWF-1	2953	142	3180	140
BRAN-1	627	80	566	71
KINO-1	5460	721	6010	720
SRM 3234	25,730	824	25,010	560
SRM 1568b	1272	21.1	1282	11
Phosphorus				
DUWF-1	2838	221	2900	220
BRAN-1	175	12	171	11
KINO-1	4231	64	ND	ND
SRM 3234	7778	230	8080	210
SRM 1568b	1562	46.65	1530	40
Copper				
DUWF-1	4.078	0.69	4.3	0.69
BRAN-1	2.87	0.5	2.47	0.4
KINO-1	6.70	0.34	ND	ND
SRM 3234	15.64	0.50	15.34	0.26
SRM 1568b	2.34	0.22	2.35	0.16
Manganese				
DUWF-1	15	1	16	1
BRAN-1	2.57	0.31	2.55	0.29
KINO-1	20.39	1.13	ND	ND
SRM 3234	36.83	1.32	36.78	0.88
SRM 1568b	19.85	1.83	19.2	1.8
Iron				
DUWF-1	39.1	4	41.5	4
BRAN-1	14.7	2.0	14.8	1.8
KINO-1	81.53	2.7	84.1	2.6
SRM 3234	82.89	3.3	80.30	2.7
SRM 1568b	6.71	1.52	7.42	0.44
Zinc				
DUWF-1	20.7	1.7	22.2	1.7

Table 4 (*continued*)

	Experimental Method		Certificate of Analysis	
	Determined value (mg kg⁻¹)	Estimated uncertainty	Certified value (mg kg⁻¹)	Expanded uncertainty
BRAN-1	19.0	2.5	18.6	2.2
KINO-1	29.33	0.83	30.6	0.8
SRM 3234	47.41	1.4	48.90	1.1
SRM 1568b	20.54	0.84	19.42	0.26
Aluminum				
DUWF-1	11.3	4.7	11.7	4.7
BRAN-1	NF		ND	ND
KINO-1	NF		ND	ND
SRM 3234	6.09	0.24	ND	ND
SRM 1568b	4.49	0.48	4.21	0.34

without causing damage or blockage of the torch during analysis. As a precaution, however, the torch was cleaned after each use.

4.2. Figures of merit

The limit of detection (LOD) was determined as 3 times the standard deviation of 10 repetitive blank measurements. The limit of quantification (LOQ) was measured as 10 times the standard deviation of the 10 blank measurements. The LOD values for Ca, Mg, K, Mn, Zn, Fe, Cu, and Al in the study ranged from 0.7 μg kg⁻¹ for Al to 598.7 μg kg⁻¹ for K (Table 3). As seen in the certified reference materials and spike recovery crop samples, the LOQ obtained from this method for each analyte is suitable for micronutrient analysis of cereals, pseudocereals, and legumes analyzed in this study (Table 3).

The calibration ranges were selected to match the expected concentrations of the CRMs and spike recovery crop samples. The calibration curves obtained for all analytes showed good linearity with a reported correlation (r^2) of 0.9979 or greater. The r^2 values (0.9979–0.9999) found in this study (Table 3) were similar to those reported in other MP-AES validation studies (Heredia et al., 2016; Ozbek et al., 2019; São Bernardo Carvalho et al., 2020).

The precision of the analytical procedure was determined by calculating the relative standard deviation (RSD). Across CRMs, the RSD (%) for Al, Ca, Mg, Fe, P, K, Zn, and Cu was 6.0, 4.8, 3.3, 4.8, 2.3, 4.2, 6.5, and 10.7 respectively. The RSD for each element was compared to the predicted RSD based on analyte concentration and determined to be acceptable for all reference materials (AOAC International, 2016).

4.3. Analytical validation

There were two analytical validations to assess accuracy during the extraction process: (1) certified reference value comparison using DUWF-1, KINO-1, BRAN-1, SRM 1568b, and SRM 3234; (2) and a spike experiment with the addition of known element concentrations (Al, Ca, Mg, Mn, Fe, P, K, Zn, and Cu) in amaranth, barley, fava, lentil, pea, maize, and lupin samples. The determined values for each analyte obtained from the MP-AES overlapped with the uncertainty intervals of the certified values for each CRM, except for magnesium in SRM 1568b matrix (Table 4). The found concentration estimates of Mg in SRM 1568b were not in agreement with the certified value, likely due to the small concentration and small uncertainty interval relative to the mean concentration reported for the CRM. Further method optimization will be needed to obtain agreement for Mg in this matrix to avoid measurement bias.

In comparison, an MP-AES validation by São Bernardo Carvalho

Table 5
Analyte recoveries (%) and RSD (%) values of analytes ($n = 7$) for all reference materials. Blank data fields indicate that CRM certificates did not have certified values for the analyte.

Element	NRC DUWF-1		NRC BRAN-1		NRC KINO-1		NIST SRM 3245		NIST SRM 1568b	
	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery
Ca	6.2	107	6.8	106	5.6	101	2.9	102	4.9	104
Mg	3.6	97	3.6	97	3.6	97	2.2	96	1.0	89
K	4.6	93	5.4	109	3.4	91	3.2	103	1.9	99
P	1.6	98	3.7	105	–	–	1.6	96	2.1	102
Mn	5.9	96	9.2	104	–	–	3.5	100	2.3	103
Zn	9.6	93	9.2	102	2.1	96	2.3	97	5.4	106
Fe	4.1	94	7.3	96	4.7	97	3.0	103	25.8	91
Cu	17.1	95	11.3	120	–	–	3.7	102	8.5	99
Al	5.6	96	–	–	–	–	–	–	10.6	107

et al. (2020) using SRM 3234 (soy flour) validated the method using recovery of certified reference values and paired *t*-tests. Although additional spike recoveries were performed to validate the method in a matrix-match sample, the method was not in agreement with certified values for SRM 3234 for potassium, zinc, and magnesium at 95 % confidence. The present study improves accuracy across the three elements.

An improvement in digestion and accuracy was observed for SRM 3234 and all reference materials when sample size was reduced (from

Table 6
Determined mean (mg kg-1) and relative standard deviation (RSD, %) of crop samples ($n = 5$) and recovery (%) values of spiked sample ($n = 3$) for cereal, pseudocereal, and legume crop samples are presented. The precision estimates were not shown to improve readability, and the RSD values ranged from 1 to 42 % depending on the element. NF = none found.

	Barley	Lupin	Amaranth	Fava	Pea	Lentil	Maize
Calcium							
Mean	510	1205	1226	1172	676	659	44
RSD	9	10	11	12	9	12	10
Recovery	100	93	91	98	95	90	104
Magnesium							
Mean	1220	2397	2440	1078	1209	1141	1154
RSD	7	6	8	8	8	8	1
Recovery	95	86	94	95	100	92	90
Potassium							
Mean	5612	12,665	5924	11,852	11,807	10,989	4153
RSD	4	5	6	4	3	6	2
Recovery	103	98	103	102	101	101	105
Phosphorus							
Mean	4151	5921	5801	2736	2518	4200	2841
RSD	5	6	6	6	6	6	4
Recovery	95	91	92	97	99	95	94
Manganese							
Mean	15.97	109	19.92	19.36	14.65	14.68	4.60
RSD	7	11	7	11	6	6	10
Recovery	96	96	95	99	89	90	98
Zinc							
Mean	32.30	59.99	34.43	52.60	45.39	39.17	19.40
RSD	7	6	7	8	8	7	6
Recovery	106	102	106	104	106	107	103
Iron							
Mean	28.57	73.53	81.98	57.67	56.29	76.88	18.91
RSD	10	9	9	7	9	8	3
Recovery	91	90	91	89	93	95	90
Copper							
Mean	4.84	9.06	6.18	12.82	7.35	10.35	NF
RSD	16	15	21	10	13	16	
Recovery	96	97	115	114	107	99	
Aluminum							
Mean	4.42	4.45	15.47	NF	5.18	16.16	NF
RSD	34	8	42		8	10	
Recovery	94	94	92		93	101	

500 mg to 250 mg), reagents doubled, and the maximum temperature increased to 210 °C. As a result, further dilutions were unnecessary, and the torch was not damaged by the high level of salt present in some of the legume samples.

4.4. Elemental analysis

The elemental range of the CRM samples (min-max in mg kg^{-1}) was Al (4.49–11.3), Ca (123–3248), Mg (797–3361), K (627–25,730), P (175–7778), Mn (2.57–36.83), Zn (19.0–47.41), Fe (6.71–82.89), and Cu (2.34–15.64). For all elements ($n = 7$), the estimated concentration fell within an 89–120 % recovery with an RSD ranging from 1.0 to 25.8 % (Table 5). With the exception of Cu in BRAN-1, the analyte recoveries for the CRMs were within the target mean recovery based on the analyte concentration (AOAC International, 2016). Fe in SRM 1568b and Cu in DUWF-1 and BRAN-1 were > 10 % RSD, suggesting further work in method optimization to reduce sample variation. Coupled with the high recovery of Cu in BRAN-1, the method may need to be adjusted for this analyte (i.e., modifying the sample uptake rate) to improve these statistics. Al, Mn, Cu, and P did not have certified values from all reference materials. Aluminum, for example, could only be tested for agreement using DUWF-1 and SRM 1568b.

Compared to ICP-OES, recovery values were similar to those reported by Araújo et al. (2002) for BRAN-1 (equivalent to NIST 8433). In this experiment and Araújo et al. (2002), the estimate of copper in BRAN-1 (found = 1.95 mg kg^{-1}, n = 3) had the highest RSD across elements (10 %). At the reported concentration in BRAN-1, the precision is expected to be low. This trend can also be seen in the present study when comparing the RSD of the principal elements (Ca, K, P, and Mg) at an average 3.6 % to trace elements (Fe, Zn, Mn, Cu, and Al) at 7.6 %.

The elemental range of the Ecuador non-spiked crop samples (min-max in mg kg^{-1}) was Al (0.72–16.16), Ca (44.45–1225.62), Mg (1096.87–2445.52), K (4153.47–12,665.32), P (2517.65–5921.32), Mn (4.60–109.49), Zn (19.40–59.99), Fe (18.91–81.98), Cu (1.82–12.82). Since the crop samples were harvested from the field, aluminum concentration can be used to test for possible soil contamination during harvest (Stangoulis & Sison, 2008). In the present study, high levels of Al were found in the amaranth and lentil samples. Conclusions regarding the nutritional quality of these samples should be made carefully because Al, Fe, and principal elements are typically found in high concentrations in the soil and seed-handling equipment components. Additional samples will need to be collected from these crops to determine a baseline Al concentration and assess sources of contamination during sample harvest and preparation.

The matrix spike recoveries for the Ecuador crop samples were used to measure accuracy through the entire mineralization step. Aside for Cu for amaranth and fava, all single analyte recoveries across crop species were within an acceptable recovery range (90 < R% > 110) (Table 6). While additional optimization may be needed for Cu, the overall results from the analyte spike recoveries show negligible analyte loss during digestion and no significant impact of the sample matrix on analyte emission sensitivities. The RSD values of the non-spiked samples using the described method ranged from 1 to 42 percent depending on the element (Table 6). The precision was lowest for aluminum, where the RSD ranged from 8 to 42 %, with the higher RSD values associated with lower precision. Although the precision was low, the recovery was acceptable. As mentioned previously, aluminum is only used to determine soil contamination of a sample where a high level of aluminum paired with high levels of iron are suspect. At a concentration of >4 mg kg^{-1} (threshold for high aluminum), precision improved to <5 % RSD.

5. Conclusions

The study demonstrated the MP-AES and microwave-assisted digestion method for multi-element determination in a diverse set of crop matrices. All crop samples were effectively mineralized using a closed-vessel microwave system with diluted acids. The method was validated using all available certified reference material from NIST and CRM, along with analyte spike recoveries of additional crops that lack certified reference material. Analyte spike recovery results suggest further method optimization is needed for quantifying copper in some matrix types. The method provides a low-cost alternative to ICP-OES for multi-element determination in cereals, pseudocereals, and legumes.

CRediT authorship contribution statement

Jessica L. Braden: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Emily F. Klarquist:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Julianne A. Kellogg:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Emily F Klarquist reports financial support, administrative support, article publishing charges, and equipment, drugs, or supplies were provided by National Institute of Food and Agriculture. Emily F Klarquist reports financial support was provided by Washington Grain Commission. Emily Klarquist reports financial support was provided by Oregon Wheat Commission. Jessica L Braden reports financial support was provided by National Institute of Food and Agriculture. Julianne A Kellogg reports a relationship with Ardent Mills, LLC that includes: employment.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101844.

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