Brazilian Journal of Analytical Chemistry
Pre-publication – Accepted after peer review
doi: 10.30744/brjac.2179-3425.TN-16-2025

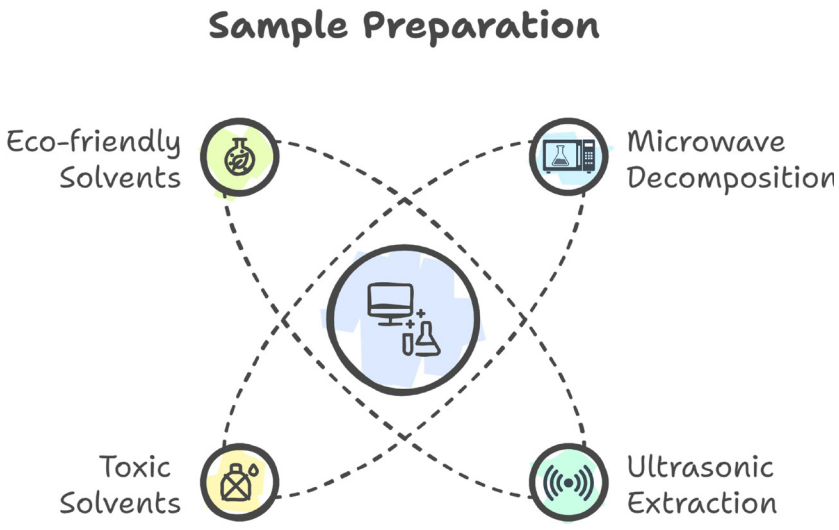
BrJAC

TECHNICAL NOTE

Sample Preparation Methods Focusing on Soy Biotechnological Samples: Towards Greener Application

João G. Veneziani Kamezawa, Lilian Seiko Kato, Elisânia Kelly Barbosa Fonseca, Marco Aurélio Zezzi Arruda*[1]

Instituto de Química, Universidade Estadual de Campinas (Unicamp). Rua Monteiro Lobato,270, 13083-862, Campinas, SP, Brazil



Tissue and cell culture techniques have become pivotal in plant biotechnology, particularly for the improvement and mass propagation of economically important species such as soybean (*Glycine max*). Within this context, somatic embryogenesis has emerged as a powerful tool. Micronutrients like Fe, Mn, Mo, and Zn play essential roles in cellular metabolism and can serve as indicators of tissue growth and development. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is widely recognized for its sensitivity in multielement analysis at trace levels; however, sample preparation remains a critical and often resource-intensive step. This study aims to develop and compare three distinct sample preparation methods for the determination of micronutrients in soybean calli using ICP-MS: (1) conventional acid decomposition, (2) acid extraction, and (3) acidless decomposition. The results indicated that all three methods provided statistically equivalent concentrations for all evaluated elements ($p \geq 0.05$), with values ranging from 0.54 ± 0.08 μg g^{-1} for Mo to 14 ± 1 μg g^{-1} for Mn. Additionally, the residual chemical content (RCC) in the proposed methods was below the detection limit of 8 mg L^{-1}. Sustainability assessment using the AGREEprep tool highlighted acidless decomposition as the most environmentally friendly method, primarily due to its use of greener reagents and higher sample throughput. These findings suggest that acidless decomposition presents a viable and sustainable alternative for micronutrient analysis in plant tissue culture applications.

Keywords: soybean calli, sample preparation, ICP-MS, decomposition, extraction

Cite: Kamezawa, J. G. V.; Kato, L. S.; Fonseca, E. K. B.; Arruda, M. A. Z. Sample Preparation Methods Focusing on Soy Biotechnological Samples: Towards Greener Application. *Braz. J. Anal. Chem.* (Forthcoming). http://dx.doi.org/10.30744/brjac.2179-3425.TN-16-2025

Submitted March 18, 2025, Resubmitted May 23, 2025, Accepted June 7, 2025, Available online June, 2025.

INTRODUCTION

The production and exportation of soy is an important pilar of the Brazilian economy.[1] Many products are derived from soybeans, such as animal feed, oil, biodiesel and others. However, it is a cheap commodity and therefore needs large production to be profitable. Because there is a limit for the cultivated area for soy and an urge to improve the production and quality of the grains, the search for biotechnological tools is an important alternative for the producer countries. A technique that has been increasingly investigated in recent times is somatic embryogenesis (SE).

SE is an important tool in plant biotechnology; it is a process in which entire plants can be regenerated form a tissue named calli.[2-7] The calli is a tissue constituted by totipotent cells,[2,5] they are formed by the dedifferentiation of cells from an explant of a plant donor. The *in vitro* cultivation of totipotent cells, able to form every part of the organism, allows many applications as genetic improvements and rapid mass multiplication of varieties.[4] Therefore, the SE can be employed for the propagation and improvement of species with economic interests.[4] However, new technical methodologies need to be developed and adapted to measure these tissues and have confinable results about their applications.

The determination of the micronutrients of the calli can be very useful as an indicator of their development. Some of the most common micronutrients are iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn).[8-10] These nutrients are essential for the cells well-functioning, even thought is necessary at low concentrations, they perform important roles for the metabolism.[9-11] Besides its importance, no work has reported the micronutrients concentration in soybean calli. For this task, the inductively coupled plasma mass spectrometry (ICP-MS) is a well stablish technique for multielement determination at low concentrations.[12-15] However, the sample preparation step is crucial for ICP-MS analysis, as the technique requires a liquid sample with low residual acidity.[12,16]

More recently, the search for greener methods has gained importance in analytical chemistry. Besides being reliable, the new methods need to be environmentally friendly. The main goals are to reduce the amount of reagents consumed, avoid the use of toxic reagents and reduce the waste and energy consumption.[17] One excellent tool to evaluate the greenness of a sample preparation method is the AGREEprep,[18] a metric tool that uses ten principles, with different weights, to analyze the method. The criteria principles are based on minimizing waste, use of safer reagents, maximizing sample throughput besides the promotion of automation, secure of the operator and others.[18]

One of the most commonly used sample preparation techniques is microwave-assisted acid decomposition. In this method, acids act under high pressure and temperature in a closed-vessel system to effectively break down the organic matter in the sample matrix. To minimize the consumption of sample material and reagents, miniaturized systems can be employed. Despite its efficiency and widespread application, this technique is associated with significant reagent use, high waste generation, and extended preparation times.

In recent years, ultrasonic extraction has gained popularity for elemental analysis. Various ultrasonic devices can be utilized, including ultrasonic probes, baths, and focused ultrasonic baths. These methods operate under milder conditions of temperature and pressure and are capable of reducing sample preparation time. However, a key limitation of ultrasonic systems is their limited sample throughput, as typically only a few samples can be processed per run.

A novel approach was introduced by Silva et al. (2024),[19] who proposed a microwave-assisted acidless decomposition method for biological samples using only hydrogen peroxide as the oxidizing agent. This technique offers several advantages, including lower blank signals and reduced residual acidity, which are particularly beneficial for subsequent analysis by ICP-MS.

Therefore, this work aims to develop and compare three different methods, a microwave-assisted acid decomposition using minivials, an ultrasonic assisted acid extraction and a microwave-assisted acidless decomposition, to prepare soy calli for the determination of micronutrients (Fe, Mn, Mo and Zn) in soybean calli by ICP-MS, taken into account the greenness of such methods, through AGREEprep[18] software evaluation.

MATERIALS AND METHODS

All the solutions were prepared with ultrapure water obtained from Direct-Q® 5 UV (Merck, Darmstadt, Germany). The ionic standards for the elements were from Fe, Mn, Mo and Zn. For the preparation methods were used sub-boiled nitric acid (14 mol L^{-1}) (Synth, São Paulo, Brazil) and hydrogen peroxide (30% m/m) (Synth, São Paulo, Brazil).

The calli samples were produced in our laboratories, according previous protocol.[20] In brief, they were induced from the cotyledon of a 3-week-old soy plant of the line Roundup Ready (RR). After the induction, the calli were transferred to a propagation culture media for 4 weeks, then, the samples were collected and storage frozen at -18 ºC. The samples were prepared in different ways for the determination by ICP-MS.

Microwave-assisted acid decomposition

The calli were ground and homogenized still frozen in a porcelain mortar. About 25 mg of the sample were added to the microwave Teflon® mini vials.[21] Then, 250 µL of sub boiled nitric acid and 100 µL of hydrogen peroxide were added. Four mini vials were put inside each conventional microwave vial with 10 mL of water.[22,23] The decomposition program was 5 min at 240 W; 5 min at 420 W; 5 min at 600 W and 15 min at 800 W, and performed at a DTG-100 Plus (Provecto Analítica, Jundiaí, Brazil). After the digestion, the samples were collected and stored. To evaluate the decomposition efficiency, the same method was employed for the preparation of a certified reference material of rice flour (NIST 1568a).

Ultrasonic-assisted acid extraction

For the extraction the same amount of calli was used and they were ground and homogenized in the same way. Then, 25 mg were added to an eppendorf tube with 250 µL of nitric acid, 100 µL of hydrogen peroxide and 150 µL of deionized water. Six flasks were attached to a plastic support that prevented them from opening during the sonication. A focalized ultrasonic bath (Cuphorn) attached to QQ700 sonicator (QSonica, USA) was used for 10 min with 60% of the amplitude in mode 1 min on/off. The maximum power achieved was around 90 W and the input energy was 50 kJ. After the extraction, the samples were filtered on 0.22 µm and storage for analysis.

Microwave-assisted using acidless method

An acidless microwave-assisted decomposition, based on Silva et al.[19] previous work, was also evaluated. The same amount of calli (25 mg) was added to the microwave minivials, in the same system as the acid digestion. The reagent used for the decomposition was only 500 µL of hydrogen peroxide. The same microwave device was employed, and the program used was 5 min at 400 W, 20 min at 790 W and 3 min at 320 W, and after the digestion the samples were collected and stored. The residual carbon content (RCC) of the decomposition was evaluated by inductively coupled plasma optical emission spectroscopy (ICP OES), using a PlasmaQuant 9100 Series (Analytik Jena, Germany). The plasma power was 1000 W, the plasma gas flow 15 L min^{-1}, the auxiliary gas flow 1.5 L min^{-1}, and the nebulizer gas flow was set at 0.90 L min^{-1}. A concentric nebulizer Micromist and cyclonic nebulization chamber were used, and the monitored wavelengths were 193.025 and 247.857 nm, both in axial mode. The calibration curve was prepared using sodium citrate (Synth, São Paulo, Brazil) from 60 mg L^{-1} to 400 mg L^{-1} of carbon.

Analytes determination

The determination of the analytes was carried out in an iCAP-TQ (Thermo Fischer Scientific, Germany), and the conditions adjusted for the performance test of the device. All the samples were diluted to have 1% acidity (v/v), and the calibration curve performed with the same acidity as well. The plasma power used was 1550 W, the plasma gas flow rate as 14 L min^{-1}, the auxiliary gas flow rate as 0.8 L min^{-1} and the nebulizer gas flow rate of 0.950 – 1.013 L min^{-1}. The introduction system was composed by a concentric nebulizer Micromist and cyclonic nebulization chamber. The monitoring mass charge ratio and the collision or reaction cell used are presented in the Table I. To avoid signal variations from the equipment, an Y connection was

added to the introduction system to inject the sample and a 10 µg L^{-1} solution of Sc, Rh or Y as internal standard. The Rh was used to normalize the measurements made in KED mode and Sc and Y to normalize measurements in TQ-O_2 mode. The monitored mass/charge ratio from the internal standard are $^{45}Sc^{16}O$, $^{89}Y^{16}O$ and ^{103}Rh.

Table I. Monitored mass charge ratio (*m/z*) and acquisition mode in the ICP-MS

	1st Quadrupole	Collision/Reaction Cell	2nd Quadrupole
Fe	NDA*	KED (He)	^{57}Fe
Mn	NDA*	KED (He)	^{55}Mn
Mo	^{98}Mo	O_2	$^{98}Mo^{16}O$
Zn	^{66}Zn	O_2	$^{66}Zn^{16}O$

*NDA – No *m/z* filtered

RESULTS AND DISCUSSION

The extraction process yielded a colorless solution containing fine particulate matter from the calli, necessitating filtration through a 0.22 µm membrane to remove residual solids. Similarly, both decomposition methods also produced clear, particle-free solutions upon visual inspection; however, for precautionary purposes, these samples were also filtered using a 0.22 µm membrane. This step is crucial for preventing particulate buildup in the ICP-MS injection system and minimizing the need for intensive cleaning between sample runs, thereby ensuring analytical consistency and instrument longevity.

As can be seen from Table II, the micronutrients were determined for the three different methods. Although there was few information about the presence of macro and micronutrients in soy calli, however, in plants, the Mo is the element that stands out because its lower concentration. This behavior can be attributed to the few numbers of proteins depending on this element.[24,25] Even at low concentrations it is important to determine Mo, since this micronutrient can alter significantly metabolism of amino acids and sugars.[25] Other micronutrients such as Fe, Mn, and Zn are present at higher concentration, around 10 µg g^{-1}. The control of Fe in the cells is also very important, because the ions Fe^{2+} and Fe^{3+} can also form reactive oxygen species (ROS).[26] Manganese plays a role in diverse process in the cells, respiration, scavenging of ROS, pathogen defense and others. Interesting, the Mn^{2+} cation can act as antioxidant specie.[27] Also, the Zn control is very important, once it is an essential metal for more than 300 enzymes, including RNA polymerases, but at high concentrations it can be toxic for plants.[28]

Table II. Micronutrients concentration (µg g^{-1}) for soy calli determined with different sample preparation methods (n=5)

Concentration (µg g^{-1})	Fe	Mn	Mo	Zn
Acid Decomposition	13 ± 1	13 ± 1	0.50 ± 0.08	9 ± 1
Acid Extraction	13 ± 1	13 ± 1	0.46 ± 0.09	11 ± 2
Acidless Decomposition	12 ± 3	14 ± 1	0.54 ± 0.08	10 ± 1

The analytes concentration determined in the samples prepared by the different methods were compared with each other, and no statistical differences were found at 95% confidence level through *t*-student test. The concentration for Fe, Mn, Mo and Zn were statistically the same for all methods ($p \geq 0.0\ 5$).

One of most common preparation technique is the microwave assisted acid decomposition.[12,29,30] The action of acid at high pressure and temperature in close vessel system decompose most of the organic matter in the matrix. Although this method is very effective and well known, it produces a large amount of waste and high use of reagents, even more it is highly time-consuming. Some alternatives have been proposed, such are the use of diluted acid,[31,32] and, in order to reduce sample and reagents the use of minivials,[21] and currently used for different proposals.[12,22,33] Ultrasonic extraction for elemental analysis has been increasing in recent years.[34-37] Many ultrasonic devices can be used, such as probe, bath or focalized bath.[36] Ultrasonic based techniques use mild conditions as temperature and pressure, moreover, can reduce the time in sample preparation.[34]

Taken into account more sustainable and green methods, recently an acidless decomposition was proposed[19] using only hydrogen peroxide as reagent. As seen in Table II, this method is equivalent to conventional methods, besides, the RCC was lower than the detection limit (8 mg L^{-1}) for the calli and 0.02% for the reference material of rice flour, indicating the efficacy of the method. Furthermore, the determination of analytes in the certified reference material was in agreement with the certified values for Fe, Mn, and Mo, with the exception of Zn (Table III). Although Zn was not accurately quantified in the certified sample, its concentration was consistent across the different methods evaluated in this study (see Table II). Additionally, the low residual chemical content (RCC) values further support the reliability and applicability of the proposed method.

An acidless decomposition pay great attention once works with a more environmentally friendly reagent, it is a clean reagent (thus providing low blanks), the production of its decomposition is only O_2 and water, the costs are absurdly low (only few cents of Dolar/decomposition). Additionally, the mechanism for its action is already explained.[19]

Table III. Micronutrients concentration (µg g^{-1}) for the reference material rice flour (NIST1568a) determined by acidless decomposition (n=4)

Concentration (µg g^{-1})	Fe	Mn	Mo	Zn
Experimental	7 ± 1	18 ± 4	1.3 ± 0.2	9 ± 1
Certified	7.4 ± 0.9	20.0 ±1.6	1.46 ± 0.08	19.4 ± 0.5
Agreement (%)	108 ± 14	90 ± 18	89 ± 11	46 ± 8

The AGREEprep tool can be employed as a metric to evaluate and compare the sample preparation methods presented in this study. This tool is based on the ten principles of green sample preparation, which emphasize minimizing analytical steps, reducing the consumption of reagents, energy, and waste, and favoring the use of sustainable and renewable materials. Additionally, it promotes maximizing sample throughput through automation, ensuring operator safety, and adopting greener analytical techniques. Each criterion is scored from 0 to 1, with values closer to 1 indicating a greener and more sustainable approach.[18]

The decomposition methods demonstrated an advantage over extraction due to higher sample throughput and the use of reusable Teflon vessels, contributing to improved sustainability. Notably, the acidless decomposition method achieved a higher overall score, attributed to the use of safer and less hazardous reagents. However, all the methods evaluated in this study received lower scores for the number of analytical steps and for relying on ICP-MS as the analytical technique, which, while highly sensitive, is not considered green due to its energy demands and complexity.

The comparations of the methods by the tool AGREEprep indicates the acidless method more adequately (Figure 1). The main advantages are the use of less hazard reagents, and less waste produced. Also, the miniatured vessels of the microwave system allow a higher sample throughput, compared to the ultrasonic method, providing a better score at AGREEprep. Besides, using reusable Teflon vessels also contributes to the score.[18]

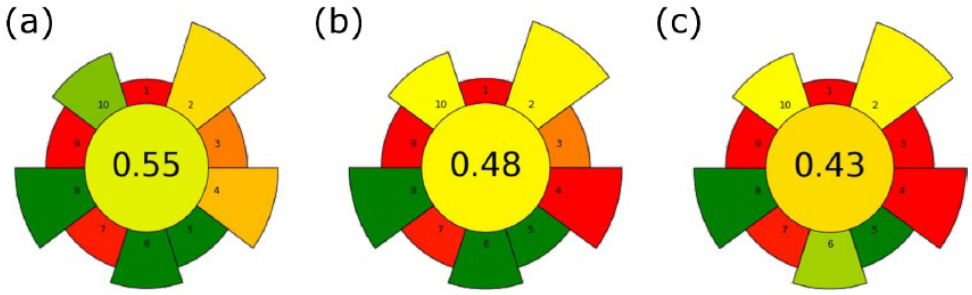


Figure 1. AGREEprep scores obtained for the acidless decomposition (a), acid decomposition (b) and acid extraction (c) methods.

CONCLUSIONS

This study evaluated three distinct sample preparation methods for the determination of micronutrients (Fe, Mn, Mo, and Zn) in somatic soybean calli. All tested methods proved to be effective and reliable for elemental quantification. The microwave-assisted acid decomposition method, widely used in analytical laboratories, demonstrated consistent performance, while ultrasonic extraction emerged as a practical alternative for laboratories lacking access to microwave systems.

However, the microwave-assisted acidless decomposition method stands out as the most sustainable option, as highlighted by its superior performance in the AGREEprep assessment. This method showed no significant difference in analytical results compared to conventional techniques, while offering clear environmental and economic advantages. It requires only a few cents (USD) per decomposition, and the exclusive use of hydrogen peroxide enhances both safety and analytical compatibility. Additionally, the use of miniaturized vials markedly reduced sample and reagent volumes, further improving the method's greenness and cost-efficiency. Collectively, these features make the acidless method a promising and eco-friendly alternative for routine micronutrient analysis in plant tissue studies.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors thank the São Paulo Research Foundation (FAPESP) (grants 2014/50867-3, 2018/25207-0, 2019/24445-8, 2021/15218-8, 2022/13166-3), National Consul of Research (CNPq) (grant number 303231/2020-3), Council for the Improvement of Higher Education Teachers (CAPES) (fellowship number 88887.653200/2021-00).

REFERENCES

(1) Flach, R.; Abrahão, G.; Bryant, B.; Scarabello, M.; Soterroni, A. C.; Ramos, F. M.; Valin, H.; Obersteiner, M.; Cohn, A. S. Conserving the Cerrado and Amazon Biomes of Brazil Protects the Soy Economy from Damaging Warming. *World Dev.* **2021**, *146*. https://doi.org/10.1016/j.worlddev.2021.105582

(2) Fehér, A. Callus, Dedifferentiation, Totipotency, Somatic Embryogenesis: What These Terms Mean in the Era of Molecular Plant Biology? *Front. Plant Sci.* **2019**, *10*. https://doi.org/10.3389/fpls.2019.00536.

(3) Midhu, C. K.; Hima, S.; Binoy, J.; Satheeshkumar, K. Influence of Incubation Period on Callus Tissues for Plant Regeneration in Ophiorrhiza Pectinata Arn. Through Somatic Embryogenesis. *Proc. Natl. Acad. Sci., India, Sect. B* **2019**, *89* (4), 1439–1446. https://doi.org/10.1007/s40011-018-01061-x

(4) Behera, P. P.; Sivasankarreddy, K.; Prasanna, V. S. S. V. Somatic Embryogenesis and Plant Regeneration in Horticultural Crops. In: Gupta, S.; Chaturvedi, P. (Eds.) *Commercial Scale Tissue Culture for Horticulture and Plantation Crops* (1st ed.). Springer Singapore, 2022, pp 197 – 218. https://doi.org/10.1007/978-981-19-0055-6

(5) Loyola-Vargas, V. M.; Ochoa-Alejo, N. Somatic Embryogenesis. An Overview. In: Loyola-Vargas, V. M.; Ochoa-Alejo, N. (Eds.). *Somatic Embryogenesis: Fundamentals Aspects and Applications* (1st ed.). Springer Cham, 2016, pp 1 – 10. https://doi.org/10.1007/978-3-319-33705-0
(6) Méndez-Hernández, H. A.; Ledezma-Rodríguez, M.; Avilez-Montalvo, R. N.; Juárez-Gómez, Y. L.; Skeete, A.; Avilez-Montalvo, J.; De-la-Peña, C.; Loyola-Vargas, V. M. Signaling Overview of Plant Somatic Embryogenesis. *Front. Plant Sci.* **2019**, *10*. https://doi.org/10.3389/fpls.2019.00077
(7) Arruda, M. A. Z.; da Silva, A. B. S.; Kato, L. S. There is Plenty of Room in Plant Science: Nanobiotechnology as an Emerging Area Applied to Somatic Embryogenesis. *J. Agric. Food Chem.* **2023**, *71*, 3651–3657. https://doi.org/10.1021/acs.jafc.2c08065
(8) Thapa, S.; Bhandari, A.; Ghimire, R.; Xue, Q.; Kidwaro, F.; Ghatrehsamani, S.; Maharjan, B.; Goodwin, M. Managing Micronutrients for Improving Soil Fertility, Health, and Soybean Yield. *Sustainability* (Switzerland) **2021**, *13*. https://doi.org/10.3390/su132111766
(9) Bagale, S. Nutrient Management for Soybean Crops. *Int. J. Agron.* **2021**, *2021* (1). https://doi.org/10.1155/2021/3304634
(10) Broadley, M.; Brown, P.; Cakmak, I.; Rengel, Z.; Zhao, F. Function of Nutrients: Micronutrients. In: Marschner, P. (Ed.). *Marschner's Mineral Nutrition of Higher Plants* (3rd Ed.). Academic Press, 2012. Chapter 7, pp 191-248. https://doi.org/10.1016/B978-0-12-384905-2.00007-8
(11) Jatav, H. S., Sharma, L. D., Sadhukhan, R., Singh, S. K., Singh, S., Rajput, V. D. An overview of micronutrients: prospects and implication in crop production. In: Aftab, T.; Hakeem, K. R. (Eds.). *Plant Micronutrients: Deficiency and Toxicity Management*. Springer Cham, 2020, pp 1-30. https://doi.org/10.1007/978-3-030-49856-6_1
(12) Bizzi, C. A.; Pedrotti, M. F.; Silva, J. S.; Barin, J. S.; Nóbrega, J. A.; Flores, E. M. M. Microwave-Assisted Digestion Methods: Towards Greener Approaches for Plasma-Based Analytical Techniques. *J. Anal. At. Spectrom.* **2017**, *32*, 1448–1466. https://doi.org/10.1039/c7ja00108h
(13) Chen, W.; Yang, Y.; Fu, K.; Zhang, D.; Wang, Z. Progress in ICP-MS Analysis of Minerals and Heavy Metals in Traditional Medicine. *Front. Pharmacol.* **2022**, *13*. https://doi.org/10.3389/fphar.2022.891273.
(14) Ahmad, R.; Shaaban, H.; Issa, S. Y.; Alsaad, A.; Alghamdi, M.; Hamid, N.; Osama, R.; Algarni, S.; Mostafa, A.; Alqarni, A. M.; Aldholmi, M.; Riaz, M. ICP-MS Determination of Elemental Abundance in Traditional Medicinal Plants Commonly Used in the Kingdom of Saudi Arabia. *Food Addit. Contam.: Part B* **2022**, *15* (2), 129–141. https://doi.org/10.1080/19393210.2022.2053591
(15) Varhan Oral, E.; Tokul-Ölmez, Ö.; Yener, İ.; Firat, M.; Tunay, Z.; Terzioğlu, P.; Aydin, F.; Öztürk, M.; Ertaş, A. Trace Elemental Analysis of Allium Species by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) with Multivariate Chemometrics. *Anal. Lett.* **2019**, *52* (2), 320–336. https://doi.org/10.1080/00032719.2018.1460376
(16) Damak, F.; Asano, M.; Baba, K.; Ksibi, M.; Tamura, K. Comparison of Sample Preparation Methods for Multielements Analysis of Olive Oil by Icp-Ms. *Methods and Protoc.* **2019**, *2* (3), 1–14. https://doi.org/10.3390/mps2030072
(17) Shi, M.; Zheng, X.; Zhang, N.; Guo, Y.; Liu, M.; Yin, L. Overview of Sixteen Green Analytical Chemistry Metrics for Evaluation of the Greenness of Analytical Methods. *TrAC, Trends Anal. Chem.* **2023**, *166*. https://doi.org/10.1016/j.trac.2023.117211
(18) Wojnowski, W.; Tobiszewski, M.; Pena-Pereira, F.; Psillakis, E. AGREEprep – Analytical Greenness Metric for Sample Preparation. *TrAC, Trends Anal. Chem.* **2022**, *149*. https://doi.org/10.1016/j.trac.2022.116553
(19) Silva, A. B. S.; da Silva Leal, K. N.; Arruda, M. A. Z. An Acidless Microwave-Assisted Wet Digestion of Biological Samples as a Greener Alternative: Applications from COVID-19 Monitoring to Plant Nanobiotechnology. *Anal. Bioanal. Chem.* **2024**. https://doi.org/10.1007/s00216-024-05472-w
(20) Silva, A. B. S.; Arruda, M. A. Z. Exploring Single-Particle ICP-MS as an Important Tool for the Characterization and Quantification of Silver Nanoparticles in a Soybean Cell Culture. *Spectrochim. Acta, Part B* **2023**, *203*. https://doi.org/10.1016/j.sab.2023.106663

(21) Flores, E. M. M.; Saidelles, A. P. F.; Barin, J. S.; Mortari, S. R.; Martins, A. F. Hair Sample Decomposition Using Polypropylene Vials for Determination of Arsenic by Hydride Generation Atomic Absorption Spectrometry. *J. Anal. At. Spectrom.* **2001**, *16* (12), 1419–1423. https://doi.org/10.1039/b107910g
(22) Santa Cruz, E. C.; Madrid, K. C.; Arruda, M. A. Z.; Sussulini, A. Association between Trace Elements in Serum from Bipolar Disorder and Schizophrenia Patients Considering Treatment Effects. *J. Trace Elem. Med. Biol.* **2020**, *59*. https://doi.org/10.1016/j.jtemb.2020.126467
(23) da Costa, L.; Arruda, M. A. Simultaneous Hydride Generation Inductively Coupled Plasma Mass Spectrometry for the Evaluation of Different Generations of Soybean Seeds. *Braz. J. Anal. Chem.* **2023**, *10* (39), 113-123. https://doi.org/10.30744/brjac.2179-3425.ar-135-2022
(24) Hänsch, R.; Mendel, R. R. Physiological Functions of Mineral Micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Curr. Opin. Plant Biol.* **2009**, *12*, 259–266. https://doi.org/10.1016/j.pbi.2009.05.006
(25) Tejada-Jiménez, M.; Chamizo-Ampudia, A.; Galván, A.; Fernández, E.; Llamas, Á. Molybdenum Metabolism in Plants. *Metallomics* **2013**, *5*, 1191–1203. https://doi.org/10.1039/c3mt00078h
(26) Lal, M. A.; Bhatla, S. C. Sulfur, Phosphorus, and Iron Metabolism. In: Bhatla, S. C.; Lal, M. A. *Plant Physiology, Development and Metabolism* (2nd ed.). Springer, Singapore, 2023, pp 351 – 357. https://doi.org/10.1007/978-981-99-5736-1_12
(27) Alejandro, S.; Höller, S.; Meier, B.; Peiter, E. Manganese in Plants: From Acquisition to Subcellular Allocation. *Front. Plant Sci.* **2020**, *11*. https://doi.org/10.3389/fpls.2020.00300.
(28) Kaur, H.; Garg, N. Zinc Toxicity in Plants: A Review. *Planta* **2021**, *253*, 129. https://doi.org/10.1007/s00425-021-03642-z
(29) Costa, L. M.; Silva, F. V.; Gouveia, S. T.; Nogueira, A. R. A.; Nóbrega, J. A. Focused Microwave-Assisted Acid Digestion of Oils: An Evaluation of the Residual Carbon Content. *Spectrochim. Acta, Part B* **2001**, *56* (10), 1981–1985. https://doi.org/10.1016/S0584-8547(01)00308-1
(30) Kwon, S. Y.; Kim, Y. I.; Kim, Y. K.; Lee, Y. B.; Mok, J. H. Microwave-Assisted Sample Preparation for Screening of Heavy Metal Elements in Food Additives by ICP-MS. *LWT* **2024**, *208*. https://doi.org/10.1016/j.lwt.2024.116708
(31) Gonzalez, M. H.; Souza, G. B.; Oliveira, R. V.; Forato, L. A.; Nóbrega, J. A.; Nogueira, A. R. A. Microwave-Assisted Digestion Procedures for Biological Samples with Diluted Nitric Acid: Identification of Reaction Products. *Talanta* **2009**, *79* (2), 396–401. https://doi.org/10.1016/j.talanta.2009.04.001
(32) Bizzi, C. A.; Flores, E. M. M.; Barin, J. S.; Garcia, E. E.; Nóbrega, J. A. Understanding the Process of Microwave-Assisted Digestion Combining Diluted Nitric Acid and Oxygen as Auxiliary Reagent. *Microchem. J.* **2011**, *99* (2), 193–196. https://doi.org/10.1016/j.microc.2011.05.002
(33) Brancalion, M. L.; Zezzi Arruda, M. A. Evaluation of Medicinal Plant Decomposition Efficiency Using Microwave Ovens and Mini-Vials for Cd Determination by TS-FF-AAS. *Microchim. Acta* **2005**, *150*, 283–290. https://doi.org/10.1007/s00604-005-0357-0
(34) Gamela, R. R.; Costa, V. C.; Pereira-Filho, E. R. Multivariate Optimization of Ultrasound-Assisted Extraction Procedure for the Determination of Ca, Fe, K, Mg, Mn, P, and Zn in Pepper Samples by ICP OES. *Food Analytical Methods* **2020**, *13* (1), 69–77. https://doi.org/10.1007/s12161-019-01524-5
(35) Coelho, T. L. S.; Silva, D. S. N.; Filho, L. B. de S.; Rocha, J. M.; de Higuera, J. M.; de Sá, I. P.; Gamela, R. R.; Nogueira, A. R. de A.; Lopes Júnior, C. A.; Vieira, E. C. High Enhancement of Macro and Micronutrients Quantification in Cajuína by ICP OES Using Ultrasound and Multivariate Analysis. *Food Chemistry Advances* **2023**, *2*. https://doi.org/10.1016/j.focha.2023.100265
(36) De La Calle, I.; Costas, M.; Cabaleiro, N.; Lavilla, I.; Bendicho, C. Use of High-Intensity Sonication for Pre-Treatment of Biological Tissues Prior to Multielemental Analysis by Total Reflection X-Ray Fluorescence Spectrometry. *Spectrochim. Acta, Part B* **2012**, *67*, 43–49. https://doi.org/10.1016/j.sab.2011.12.007
(37) Manjusha, R.; Shekhar, R.; Kumar, S. J. Ultrasound-Assisted Extraction of Pb, Cd, Cr, Mn, Fe, Cu, Zn from Edible Oils with Tetramethylammonium Hydroxide and EDTA Followed by Determination Using Graphite Furnace Atomic Absorption Spectrometer. *Food Chem.* **2019**, *294*, 384–389. https://doi.org/10.1016/j.foodchem.2019.04.104