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Assessing the greenness of optimized ultrasound and infrared assisted extraction through analytical metrics

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ABSTRACT

Sample preparation involves a critical step due to the high organic matter content that implies its decomposition, prior to analysis. Two alternative analytical strategies for sample preparation employing diluted HNO₃ were optimized and compared, assisted by ultrasound (USAE) and infrared (IRAE) radiation. Multielement determination was achieved by MIP OES. For optimization of experimental conditions, a central composite design centered on the faces was carried out, considering four and five factors for IRAE and USAE, respectively. Dissolved organic carbon (DOC), residual acidity (RA) and solid residue (SR), significantly influence the responses to be evaluated. The response surface methodology was implemented to find the best combination of mass, diluted reagents (HNO₃ and H₂O₂), time and temperature in order to minimize responses for elemental extraction in animal feed samples. The optimized experimental conditions: 500 mg of sample mass, 60 °C of bath temperature, 30 min of extraction time, 2 mol L⁻¹ HNO₃ and 27% H₂O₂, were used to validate the USAE procedure. The conditions established for IRAE were 500 mg of the swine feed sample, followed by the addition of 2 mL of 2 mol L⁻¹ HNO₃ and 23% H₂O₂, irradiating for 23 min. Recoveries ranged from 81 to 114%, with good precision (RSD < 4%). Using five index tools the greenness of the proposed methods was demonstrated. According to IRAE simplicity, reliability, fast and easy to implement, it was successfully applied to the determination of Ca, Cu, Fe, K, Mg, Mn, P and Zn in agrifood samples.

Keywords: *Green tools, Infrared assisted extraction; Ultrasound assisted extraction; Microwave induced plasma atomic emission spectrometry; Multivariate optimization*

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1. Introduction

Sample transformation into a suitable form for the subsequent multielemental determination by plasma-based techniques, such as inductively coupled plasma optical emission spectrometry (ICP OES), microwave induced plasma optical emission spectrometry (MIP OES) or inductively coupled plasma mass spectrometry (ICP-MS) [1,2], is a significant issue of the analytical process. Complex matrix of samples can cause problems during analyzes related to spectral and/or physical interferences [2]. Thus, sample treatment methods involving concentrated mineral acids and oxidants, such as nitric acid (HNO_3) and hydrogen peroxide (H_2O_2), high temperatures and high pressures have been presented as a good alternative for this purpose, being the most time-consuming step in the analysis [3].

In the study of approaches to improve reagent blanks, limits of detection (LOD) and quantification (LOQ), waste and equipment damage, greener methods using milder conditions have been set in recent years [4]. Also, more economical, safer and with less toxic waste generation analytical methods have been proposed in agreement with the 12 principles of Green Analytical Chemistry (GAC) [5,6]. On this way, several metric tools have been established to evaluate and measure the greenness of the methods using the GAC concept by the combination of scores or graphics: Analytical Eco-Scale (AES) [7], National Environmental Methods Index (NEMI) [8], Green Certificate (GC) [9], Green Analytical Procedure Index (GAPI) [10], Pictograms like Hexagon (H) [11], Analytical Greenness Calculator (AGREE) [12], and recently developed White Analytical Chemistry (WAC) approach [13]. Particularly, analytical greenness metric for sample preparation (AGREEprep) [14,15] could be used for sample pretreatment step, evaluating 10 principles of Green Sample Preparation (GSP) [16]. GSP differs from GAC in that its principles focus

on sample preparation. The first could be considered a comprehensive and systematic improvement of the greenness sample preparation methods. Direct measurement is desired for GAC, however, as is known, this is not possible in many cases, and pretreatment is required [15–17].

Thus, in recent years there has been a growing demand for green sample preparation methods. Ultrasonic (US) energy could be considered as an alternative for analyte extraction since ultrasound generates an auxiliary energy promoting the interaction between sample elements and the acid extracting media [18], transferring analytes from the organic matrix to aqueous phase. Through solid-liquid ultrasound assisted extraction (USAE) the contact surface area between solid and liquid phase is increased, allowing larger solvent penetration into the sample matrix [3,19,20]. Analyte extraction follows the principle of cavitation through the application of US waves in a medium, usually water. Expansions and compression cycles can locally cause temperatures up to 5000K and pressures up to 1000 atm. In these alternating high-pressure / low-pressure cycles, gasses and molecules in the vapor phase can be expelled from bubbles [3,20,21].

Another way to prepare samples for inorganic species determination is through solid–liquid infrared assisted extraction (IRAE). Due to the ability to promote faster heating, the infrared (IR) radiation was explored in different systems. As a heating source, it has found many applications (mainly in the purification of acids by sub-boiling distillation) [22], and more recently in wet digestion for sample preparation [23–26]. The heating mechanism is based on the IR radiation's deep penetration into the substances. Specifically, molecules absorb radiation, stimulating molecular vibration that directly produces heat [23,27].

Hence, USAE and IRAE could be considered simple, inexpensive, rapid, robust, and an environmentally friendly alternative to conventional extraction techniques. However, their disadvantage is related to the size and design of the ultrasonic bath and infrared digester, which define the quantity of processed samples at once [20]. The aim of this work was to develop and assess the greenness of the USAE and IRAE procedures through five analytical metrics. A central composite design was used to optimize the process variables (sample mass, HNO_3 and H_2O_2 concentrations, time and temperature). The optimized procedure makes sample preparation faster, environmentally friendly and with a higher throughput. It was applied to multielemental analysis of swine feed samples by MIP OES.

2. Experimental

2.1. Reagents and solutions

All solutions and sample dilutions employed were prepared using high purity water (18 $\text{M}\Omega\text{ cm}^{-1}$ resistivity) produced from a Milli-Q purification system (Millipore® ultra-purifier, Darmstadt, Germany). All glassware and propylene vessels were previously cleaned by soaking them in a HNO_3 10% (v v^{-1}) solution for 24 h and rinsed with ultrapure water before use. The standard solutions used for the external calibration were prepared by appropriate dilution of a multielemental standard SCIENCE Plasma CAL (1000 mg L^{-1} with 5% nitric acid) for the determination of Ca, Cu, Fe, K, Mg, Mn, P and Zn.

Concentrated HNO_3 (65% w w^{-1} Merck, Darmstadt, Germany), previously purified using a Berghoff® sub-boiling distiller system (Eningen, Germany), and H_2O_2 (30% w w^{-1} Sigma-Aldrich) were used for sample preparation.

Oxalic acid (Mallinckrodt, Paris, KY, USA) as carbon (C) source was dissolved in 1% HNO_3 to obtain a standard 1% C stock solution, which was sequentially diluted to obtain

the C calibration solutions (0.05 to 1%) for dissolved organic carbon (DOC) determination. Carbon was determined by MIP OES using the atomic emission line 193.027 nm. Residual acidity (RA) determination of the digests was carried out titrating with NaOH (Merck, Germany) 0.0201 mol L⁻¹, previously standardized using potassium hydrogen phthalate (C₈H₅KO₄, Merck, Germany).

2.2. Samples

The optimized procedure was applied to the analysis of agrifood samples from different local farms of La Pampa, Argentina. Samples were collected in polypropylene metals free containers, and kept in darkness. Six samples from different physiological stages were employed: growth (2 samples), development (2 samples), gestation and lactation. The samples were dried at 60 °C for 24 h and grinded using conventional grinding equipment with liquid nitrogen.

In addition, a material of a proficiency test produced by EMBRAPA Pecuária Sudeste (São Carlos, SP, Brazil): Ground Corn (MRC 20), was used to evaluate the efficiency of sample preparation methods.

2.3. Instrumentation

A MIP OES Agilent MP 4210 with axially-viewed configuration (Santa Clara, USA) was used for Ca, Cu, Fe, K, Mg, Mn, P and Zn determination. The spectrometer includes a Czerny-Turner monochromator with a VistaChip charge-coupled device (CCD) array detector, operating on line with a nitrogen generator. A single-pass cyclonic spray chamber, a One Neb inert nebulizer, an SPS3 auto-sampler system and a quartz plasma torch

(Agilent, Australia) were used. The operational parameters and emission lines wavelengths selected for all analytes are presented in Table 1.

Two energy sources were employed to facilitate the target elements' extraction. USAE method was carried out using an ultrasound bath (Testlab, Buenos Aires, Argentina) with a frequency of 40 kHz and a power of 160 W. The temperature of the filling water bath tank was regulated in the range from 30 to 60 °C, while the sonication time was changed within 15–45 min. For the IRAE an IR heating digester prototype was used [23]. This prototype is equipped with two commercial IR lamps (250 W, France, China) and with a fixed maximum operating temperature of 190 °C, approximately.

2.4. Multivariate optimization of the extraction procedures

A full central composite design with face-centered central points (FC-CCD) was used to study the effect of the four and five variables for IRAE and USAE, respectively. Sample mass (100-500 mg), extraction time (15-45 min), HNO₃ concentration (2-7 mol L⁻¹), H₂O₂ concentration (10-30%) were evaluated for both experimental designs, while bath temperature (30-60 °C) was only evaluated for USAE. Three replicates at the central point were made in order to evaluate the experimental error. To ensure the independence of the results and to minimize the effects of uncontrolled factors all experiments were performed in random order.

With the aim to find the improved conditions that ensure the optimal compromises among all responses [20], the experimental parameters were optimized by minimizing the response of dissolved organic carbon (DOC), residual acidity (RA) and solid residue (SR) applying the desirability (D) function.

The results of experimental design are presented in Tables S1 and S2. The statistical analysis for each response in both designs were fitted to different models calculated by backward multiple regression and validated by analysis of variance (ANOVA) tests ($p \leq 0.05$). The model adequacy was evaluated by the coefficient of determination (R^2), coefficient of variation (CV) and the model p-value. These experimental data were processed using well-known routines implemented by the statistical software Design Expert 11 (including in Unscrambler X software, CAMO, Trondheim, Norway). In order to validate the results, three additional experiments under the optimal conditions were conducted and confirmed for each design.

2.5. Statistical analysis for evaluation and validation of quantification models

Linearity, LODs and LOQs, and repeatability were used to assess and validate the proposed green methodologies. The values of LOD and LOQ were computed according to IUPAC recommendations, equations (1) and (2) [28]:

$$LOD = \frac{3.3S_{y/x}}{A} \sqrt{1 + h_0 + \frac{1}{I}} \quad (1)$$

$$LOQ = \frac{10S_{y/x}}{A} \sqrt{1 + h_0 + \frac{1}{I}} \quad (2)$$

where $S_{y/x}$ is the residual standard error of the calibration curves, A is the slope, h_0 is the leverage for the blank and I is the number of calibration samples.

Repeatability and recoveries (95% confidence level) were calculated based on analyzed proficiency materials. All the experiments were carried out with a minimum of three replicates. Reagent blanks were performed. Data analysis was carried out using Microsoft Excel® 2007.2.4.2, considering a 95% confidence level.

3. Results and discussion

3.1. USAE optimization

To improve the extraction process, multivariate optimization was applied. It allowed evaluation of variables affecting analyte extraction and their interactions to obtain optimal conditions enabling less experiments compared to univariate methods [29,30]. An animal feed sample was selected to optimize the USAE. The forty seven (47) experiments were presented in Table S1, as well as the percentages of DOC, RA and SR, being these three parameters significant in order to evaluate the OM oxidation efficiency.

The percentage ranges were 0.024–0.193% for DOC. Quantification of DOC in the solution after extraction shows the C quantity that will be introduced in the equipment and allows inferring the decomposition efficiency [3]. This is relevant due to soot deposition on the MIP torch and the spectral and non-spectral interferences caused by carbon-based molecular bands emission in elemental determination by MIP OES [31].

Values of RA ranged from 1.7 to 8.7% for the US extractions. The final acidity in extracts is represented by RA and must not exceed 5% ($v v^{-1}$) for MIP OES determination [32], nevertheless 32% of the evaluated extracts exceeded this value (Table S1). In this sense, a reduction in the signal can be observed compared to water by changes in the physical properties of the solution and variations in aerosol generation process, modifying the analyte mass that is carried to the plasma [33–35].

Additionally, SR presented values ranging from 11.2 to 61.9%, being between 4 and 21 times higher than the recommended value of 3% [32].

Response Surface Methodology (RSM) -based on FC-CCD and D function approaches- was used as strategy to optimize all variables at the same time with multiple responses (DOC, RA and SR) [20,36]. The experimental data provided by the FC-CCD were analyzed by regression analysis. ANOVA was implemented to evaluate the fitted mathematical model with a statistical significance at $p \leq 0.05$, analyzing the R^2 , F-values for the lack-of-fit and the CV (Table 2). Quadratic and linear models did not present a lack of fit demonstrating that the models were highly adequate and significant. Moreover, the CV values were lower than 10% showing that the variation is statistically acceptable and satisfactory. As mentioned, the best experimental conditions were performed by RSM, using the D function to simultaneously assess the multiple responses. Figure 1 shows the response surfaces of D, displaying the relationship between two factors, as well as their effects and interactions.

In Fig. 1.e, 1.f, 1.i and 1.j, it is evident that the use of lower HNO_3 concentrations promotes more efficient extractions; while the same effect, although less pronounced, occurs when higher H_2O_2 concentrations are employed (Fig 1.b, 1.g and 1.j). Both reagents are attractive for OM decomposition according to the GAC and GSP. On the one hand, diluted HNO_3 is advantageous regarding lower blanks, less equipment damage and interference reduction. On the other hand, H_2O_2 could be considered according to green chemistry recommendations as the main decomposition products are O_2 and H_2O [35]. In Fig. 1.a, 1.b and 1.e the larger sample masses effect is depicted, which is profitable in order to obtain better LODs.

In summary, USAE is favored when mass, temperature and H_2O_2 concentration increase to upper levels, whilst HNO_3 diminishes at the lower level. The optimized

experimental conditions for the achieved maximum D value ($D = 0.74$) were: 500 mg of sample mass, 60°C of bath temperature, 30 min of extraction time, 2 mol L⁻¹ HNO₃ and 27% H₂O₂.

Optimized conditions were used to validate the USAE procedure for swine feed samples. Three additional experiments were conducted. Considering the predicted mean values (and their respective standard deviation of the prediction-SDP-), optimal conditions were confirmed with a confidence level of 95 %. The achieved values were: 1.99 (0.28) for RA, 33.42 (1.26) for RS and lower than LOD for DOC, which did not show significant differences with the theoretical results (data not shown).

3.2. IRAE optimization

In IRAE several variables (individually and/or collectively) such as extractor type and concentration, time, sample mass, among others [36] could affect the extraction. Thus, factor optimization is a mandatory step. The effects of four parameters described in 2.4 were investigated and optimized using FC-CCD and RSM. The FC-CCD consisted of twenty seven (27) experiments including three center points. For IRAE extraction, the percentage ranges were: 0.024–0.171% for DOC, 1.6– 8.0% for RA, and 6.5–35.5% for SR (Table S2).

As could be seen, the range for DOC is similar in both extraction procedures, USAE and IRAE. For RA, 55% of the extracts exceed or are within the limit of 5% established [34,35]. Finally, as for USAE optimization, all solutions exceed the recommended 3% of SR, choosing for this reason a filtration step.

ANOVA was used afresh to assess each studied variable significance. The obtained models did not show lack of fit. The resulting R^2 , F-values for the lack-of-fit and CV indicate that the experimental data were in satisfactory agreement with predicted responses for each model, evidencing the fitness of the model (Table 2). The response surface plot is presented in Figure 2.

Results showed that high sample masses and lower HNO_3 concentrations resulted in high D (Fig. 2.b). Figure 2.d shows the combined effect of HNO_3 concentration and time, while Figure 2.f illustrates the combined effect of HNO_3 and H_2O_2 concentrations. Both figures presented maximum regions when more diluted acid is used. This is attractive due lower waste generation, lower costs, less damage in the equipment, among others discussed in the previous section. For H_2O_2 concentration, Figures 2.c y 2.e show the combined effect with sample mass and time respectively. Thus, higher H_2O_2 percentages combined with lower times and higher masses promote better OM decomposition. The larger sample masses effect is depicted in Fig. 1.a, 1.b and 1.c.

To sum up, the maximum desirability value ($D = 0.65$) was obtained for the conditions established for IRAE of 500 mg of the swine feed sample, followed by the addition of 2 mL of $2 \text{ mol L}^{-1} \text{HNO}_3$ and 23% H_2O_2 , irradiating for 23 min.

Optimal conditions were experimentally assessed through three replicates obtaining mean values (and their respective standard deviation). For RS and RA, the values were 1.97 (0.40) and 23.58 (1.05), respectively; for DOC they were lower than LOD. Experimental results did not show significant differences with theoretical results.

3.3. Analytical performance

The parameters studied during the validation processes (linearity, LOD, LOQ, trueness and precision) are depicted in Table 3. The calibration curves were constructed at five levels in triplicate, obtaining good linearities with regression coefficients (R^2) higher than 0.996 (Table 3) for all analytes. The LOQs ranged from 0.19 to 75 mg kg⁻¹ for Cu and P respectively, and could be considered adequate for elemental determination in agrifood.

A proficiency material (corn grain with similar animal feed matrix) was analyzed. As can be seen in Table 3, the recoveries ranged from 86 to 114% for IRAE, and 81 to 99% for USAE with exception of Zn with 132% (indicating possible contamination). The obtained RSD values ranged from 0.2 to 4% for all analytes (Table 3), indicating a suitable precision (values lower than 10%). The results denote excellent extraction efficiency for eight elements by IRAE (Ca, Cu, Fe, K, Mg, Mn, P and Zn) and seven by USAE (Ca, Cu, Fe, K, Mg, Mn and P).

3.4. Environmental impact assessment

It is well known that sample preparation is the most time consuming and most likely contamination step. Thus, with a huge relevance in the greenness of analytical methods, especially for complex matrices [3]. Considering GAC principles, procedures without sample treatment are ideal. However, the elimination of sample treatment is still a challenging task for many foodstuff samples. In this way, the reduction of energy and reagents consumption, as well as the number of stages is sought.

In order to evaluate the greenness and reasonable ranking for both developed methodologies, a comprehensive study of green metrics was carried out. Five different tools were used for this purpose, including AES, GC, H, AGREE and AGREEprep.

The AES -described by Galuszka et al. [7] could be considered one of the most simple numerical score metrics. It is based on the penalty points (PP) deduction of four parameters (reagents amount and hazard, consumed energy and generated waste) on a 100 points basis. Following this criteria, the methods could be classified in three categories as: excellent (>75), acceptable (50-75) and inadequate (<50) [7]. According to this tool, the final score for both, USAE and IRAE, is 79. This means they are classified as excellent green procedures with minimal negative impact on the environment. Table 4 (and Table S3) shows the AES total score for both analytical methodologies. The higher PPs subtraction for reagents (HNO_3 and H_2O_2) made the process less green. Despite this, AES provides an easy comparison by means of method's greenness quantitative valuation. AES does not consider the reagents pictograms, it only considers the waste quantity, not its nature.

Also USAE and IRAE were compared concerning GC, which could be viewed as an AES modification. Weighted penalty points are assigned for GC, taking into account reagents, consumed energy, human hazards and generated wastes. It establishes a scale from A to G using a scale of seven colors associated (from green to red). The greenest methodology is associated with a green color (A), while the red color (G) corresponds to a not sustainable methodology [5]. The total scores obtained for GC were 90.4 (A) and 90.6 (A), for USAE and IRAE, respectively (Table 4 and Figure S1), showing the eco-friendliness of both methodologies. No significant differences were observed between them, but the main difference is related to the higher PPs for the optimal H_2O_2 concentration, in USAE procedure. One advantage of GC is that it employs equations to quantify the reagents and waste PPs.

The H tool is one of the most interesting and complete metric proposed. It is composed of six equilateral triangles. Similar to AES and GC, PPs are assigned to calculate the score of the metric based on five variables: figures of merit (two triangles –yellow and blue-), environmental friendliness (green triangle), generated residues (grey triangle), economic cost (red triangle) and toxicity-safety included in the same triangle (brown triangle). For each triangle, the final score is scaled from 0 to 4, where the scale indicates excellent (0), good (1), suitable (2), weak (3) and fail (4) performance [11]. The great advantage of this metric is costs and analytical performance evaluation, involving sample preparation, method characterization, calibration, quality control and accuracy. Also, the generated residues calculation is improved and the environmental impact is quantified by the carbon footprint metrics. This metric provides advantages for greener assessing, covering more analytical procedure aspects than AES and GC. However, developing software to introduce the information and speed up the calculations would be highly useful due to the many tables employed.

For USAE and IRAE optimized methods, H pictogram data revealed 2 zeros, 4 ones and 1 two as scores (Table 4). This could be considered concordant with the GAC principles. USAE and IRAE showed differences in the carbon footprint, related to energy used per sample. IRAE required less energy during sample preparation step due to the processing capacity of 47 samples per hour, while USAE is capable of treating 18 samples per hour. Added, the energy consumed by the IR lamps is less than the energy consumed by the thermostated US bath. The results obtained for both procedures do not show green differences with AES, GC and H, verifying the worthy performance of both techniques.

Nowadays, the most popular free software metrics to assess the greenness of an approach are AGREE and AGREEprep. They allow obtaining a score ranging between 0-1 together with a color-coded pictogram (red-yellow-green color scale). The final score arises from mathematical functions that refer to 12 GAC principles (12 input variables) and 10 GSP basis (10 input variables) for AGREE and AGREEprep, respectively [12,14]. The higher scores (green-tinted tones) could be considered greener while low scores correspond to less green methodologies (red-tinted tones). AGREE could be considered a straightforward green metric which provides an informative and easily interpretable result. The overall AGREE scores are represented in Table 4, obtaining a value of 0.51 for both proposed methods (the detailed scores are presented in Table S4). Despite not showing differences in the metrics evaluated, IRAE provides high throughput, low H₂O₂ and energy consumption, compared to USAE.

Regarding AGREEprep, some weights were modified based on the developed methodologies. The weight of the input 9 was adjusted to 5 considering that MIP OES does not consume gasses. It uses atmospheric air to obtain the N₂ that generates the plasma. Also as IRAE and USAE use diluted reagents, the weight for input 10 was set to 1. The AGREEprep scores for USAE and IRAE were 0.46 and 0.49 (Table 4 and detailed scores in Table S5), respectively. The results evidence differences for both procedures in the sample treatment. It is important to highlight that the previously evaluated metrics do not focus specifically on the sample preparation stage, as this one does.

Green metrics are becoming increasingly important to obtain an integrative and informative classification of a method, but currently a single metric to assess the greenness is not enough. Tools such H and AGREE present many aspects considered to be similar

(AGREE points 2, 3, 5, 7, 8, 9, 11 and 12). The AGREEp_{rep} tool also comprehensively includes sample treatment and preparation. Thus, it is important to emphasize that in the free software metrics, it would be valuable to add some attractive aspects that are considered in the H tool, as the merit figures, including calibration, precision and quality control.

3.5. Swine feed samples multielemental analysis

Monitoring the mineral composition of animal feed is an important criterion for the assessment of product quality [37]. Essential elements (Mg, P, K, S, Na, Co, Cu, Fe, Mn, Zn, Ca and Se) in adequate concentrations are vital for physiologic and biochemical functions, while other elements can be toxic at relatively low concentrations [1,38,39]. Nutritional requirements are adapted to different animal species needs and their varying life stages, thereby, it is critical to determine elemental composition in foodstuffs [38].

Certain aspects allow opting for one procedure over another. Although both methodologies present many similarities when comparing their green indices (section 3.3), USAE allows the determination of one less element when considering the recoveries obtained (section 3.2). Likewise the sample throughput of IRAE is greater. Another aspect in which IRAE stands out is the lower energy consumption and lower consumption of one of the reagents (section 3.1 and 3.3). In light of these advantages, analytical application was carried out using IRAE.

Six swine feed samples used in farms from La Pampa province (Argentina) were submitted through the proposed method under optimized experimental conditions and the results are shown in Table 5. The Ca and P concentrations for growth and development

stages were close to the recommendations proposed by National Research Council (NRC) [40]. Concentrations of Fe for three stages (Development II, Gestation and Lactation) were lower than the reference values. It would be necessary to be cautious since its deficiency causes anemia [40]. Concentrations of Cu were above the recommendation, which demonstrated the need to control this element in animal feeds as it could be toxic in excess of 250 mg kg⁻¹ for extended periods of time [40]. Others analyzed elements such as K, Mg and Mn present concentrations higher than those recommended by the NRC in all stages of pig development. However, no toxic effects are known for K and Mg, whilst for Mn depressed feed intake, reduced growth rates and reduced hemoglobin levels have been reported [40]. The found Zn concentrations were above those recommended. Since its toxicity depends on the source, dietary level, feeding duration and the presence and level of other minerals in the diet, its effect would have to be evaluated [40]. Also, excessive nutrients could be considered costly and contribute to environmental pollution.

4. Conclusion

Two useful, simple and efficient sample preparation procedures (USAE and IRAE) have been developed and validated in the present study. Both offer an effective sample preparation method for Ca, Cu, Fe, K, Mg, Mn, P and Zn (the last only for IRAE) determination in animal feed by MIP OES. Under optimized conditions, they showed significant precision and trueness, as well as adequate analytical characteristics, such as LODs, LOQs and linearities.

In addition, the procedures show significant advantages due to the use of low and diluted reagents, low cost instruments, high sample throughput (IRAE), less acidic waste

among others aforementioned. These features are in accordance with GAC and GSP recommendations. The obtained results confirm that the methods (USAE and IRAE) are worthwhile green sample preparation approaches to assess the quality of swine feed samples demonstrated by applying five green indices.

Declaration of interests

- ✓ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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TABLES

Table 1. Operational conditions for determination by MIP OES.

MIP OES instrument parameters	Operational conditions
Plasma power (kW)	1.0
Stabilization time (s)	10
Background correction	Auto
Integration time (s)	3
Nebulizer	OneNeb® concentric
Spray chamber	Single pass cyclonic
Sample flow rate (rpm)	15
Replicates	3
Analytes	Ca (393.366 nm), Cu (24.754 nm), Fe (371.993 nm), K (766.491 nm), Mg (285.213 nm), Mn (403.076 nm), P (213.618 nm) and Zn (481.053 nm).

Table 2. Model fitting for each analytical response and obtained statistical values when applying ANOVA of lack of fit and regression of the selected models.

Extraction	Response [%]	ANOVA p-value		R-squared	CV [%]
		Model ^a	Lack of fit ^a		
USAE	DOC	Quadratic	0.6780	0.99	8.93
	RA	Linear	0.7499	0.96	5.03
	SR	Quadratic	0.5273	0.99	4.50
IRAE	DOC	Quadratic	0.9087	0.95	5.86
	RA	Linear	0.6505	0.96	8.28
	SR	Quadratic	0.5158	0.98	6.52

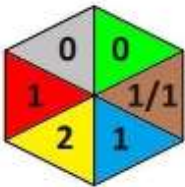
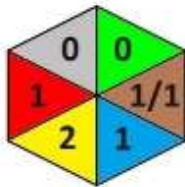
^a p-values less than 0.05 indicate significance.

^b % = $\frac{SD}{X} \times 100$. Coefficient of variation is the error (standard deviation-SD-) expressed as a percentage of the mean (X).

Table 3. Analytical parameters obtained for each validated element.

Analyte	LOD	LOQ	USAE Recovery	IRAE Recovery	RSD	R ²
	[mg kg ⁻¹]	[mg kg ⁻¹]	[%]	[%]	[%]	
Ca	1.2	3.6	96	101	4	0.996
Cu	0.06	0.19	94	114	0.4	0.999
Fe	5	16	93	107	3	0.998
K	1.4	4.3	81	90	2	0.997
Mg	2.4	7.3	90	107	2	0.999
Mn	0.68	2.05	91	86	0.2	0.999
P	22	75	99	102	0.3	0.999
Zn	1.11	3.35	132	88	2	0.999

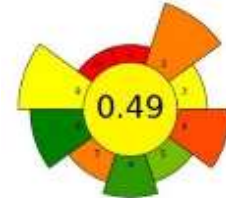
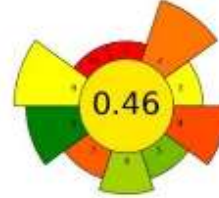
Table 4. Comparison between the five evaluated metrics for analytical procedures employing US and IR as energy source for extraction.

Metric tool	US	IR
AES	79	79
GC	90.4	90.6
H		

AGREE



AGREEprep



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Table 5. Macro and micro elements in swine feed samples (mean \pm standard deviation, n = 3) using optimized IRAE

	Ca		Cu		Fe		K		Mg		Mn		P		Zn	
	[mg kg ⁻¹]															
Growth stage I	5165	\pm 524	83.1	\pm 3.4	115.4	\pm 10.5	8169	\pm 397	1403	\pm 74	40.9	\pm 6.4	5289	\pm 157	76.7	\pm 6.0
Growth stage II	5432	\pm 262	94.4	\pm 7.6	88.1	\pm 3.8	8834	\pm 165	1744	\pm 23	73.9	\pm 4.0	5219	\pm 133	136.3	\pm 3.8
Development phase I	5747	\pm 327	108.6	\pm 6.2	117.8	\pm 1.8	7776	\pm 241	1397	\pm 32	44.8	\pm 0.6	5308	\pm 117	97.9	\pm 7.1
Development phase II	4870	\pm 797	107.3	\pm 6.9	34.2	\pm 11.3	7822	\pm 78	1506	\pm 16	45.6	\pm 3.5	4255	\pm 87	97.9	\pm 12.2
Bristle in gestation	6424	\pm 981	17.6	\pm 9.6	61.9	\pm 1.8	5650	\pm 237	1343	\pm 74	101.0	\pm 57.7	3530	\pm 146	137.8	\pm 6.1
Bristle in lactation	6712	\pm 236	16.6	\pm 5.4	51.4	\pm 7.4	8508	\pm 422	1663	\pm 68	84.1	\pm 22.9	4368	\pm 241	136.5	\pm 5.0

FIGURES

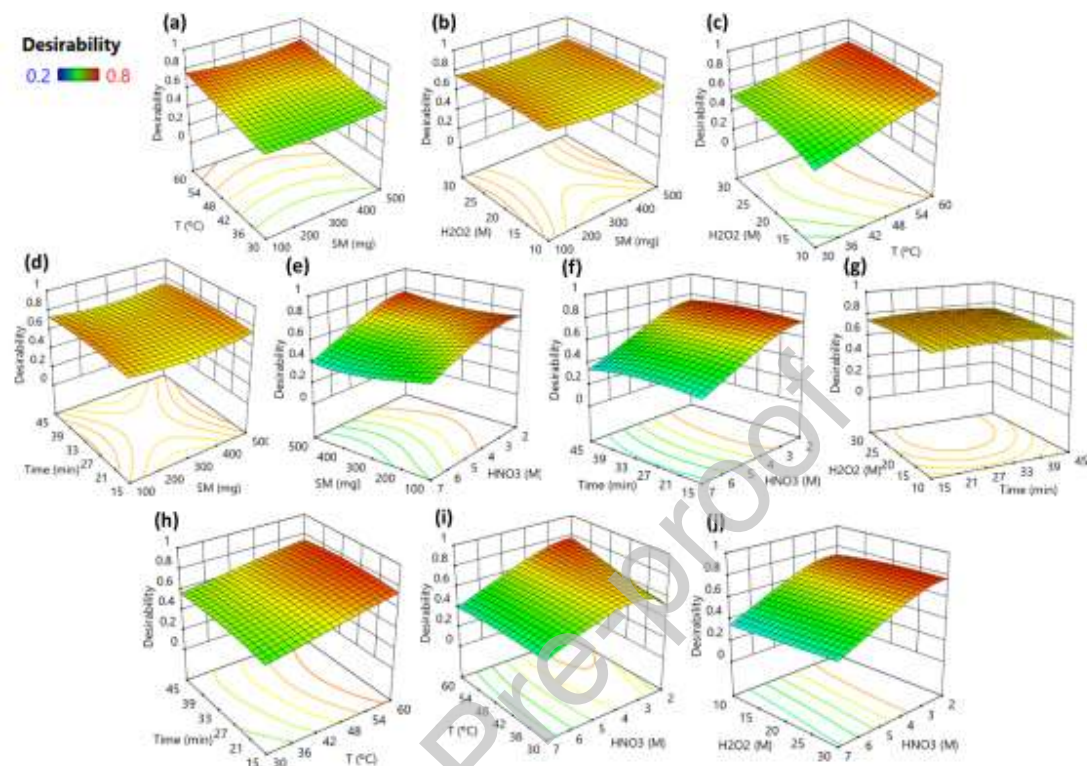


Figure 1. Response surfaces of the desirability function representing the relation between two different factors: (a) temperature and sample mass (SM); (b) H_2O_2 concentration and SM; (c) H_2O_2 concentration and temperature; (d) time and SM; (e) SM and HNO_3 concentration; (f) time and HNO_3 concentration; (g) H_2O_2 concentration and time; (h) time and temperature; (i) temperature and HNO_3 concentration; (j) H_2O_2 concentration and HNO_3 concentration.

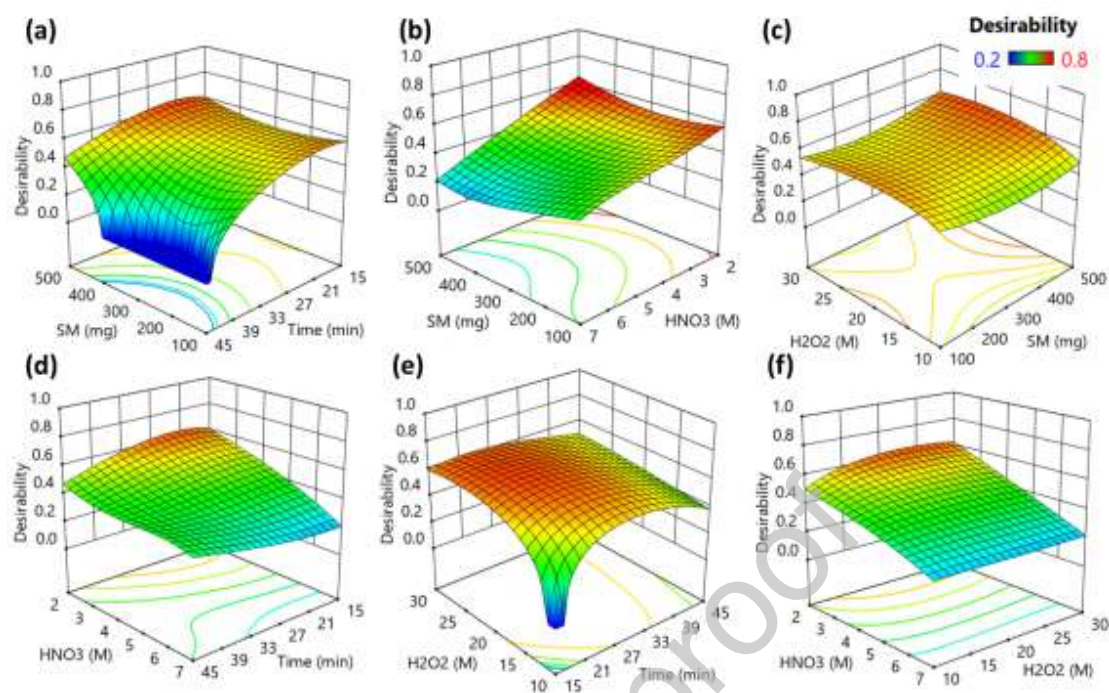


Figure 2. Response surfaces of the desirability function representing the relation between two different factors: (a) sample mass (SM) and time; (b) SM and HNO₃ concentration; (c) H₂O₂ concentration and SM; (d) HNO₃ concentration and time; (e) H₂O₂ concentration and time; (f) HNO₃ concentration and H₂O₂ concentration.



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