

Comprehensive Optimization of Nitric Acid and Hydrogen Peroxide in Microwave-Assisted Digestion for Simultaneous Multi-Mineral Extraction in Diverse Food Matrices

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Abstract

This study aimed to systematically optimize microwave-assisted digestion parameters (HNO_3 and H_2O_2 concentrations) for the simultaneous extraction of 13 major and trace minerals in food samples using Response Surface Methodology (RSM). To systematically evaluate the extraction behavior, a Standard Reference Material (SRM) multivitamin/multielement tablet was utilized as a baseline. Against this reference, three complex food matrices—chocolate, cheese, and nori—were comprehensively investigated, having been specifically selected to represent diverse and challenging protein-fat-carbohydrate (P-F-C) profiles. The adequacy and predictive accuracy of the constructed models were comprehensively validated using statistical evaluations, including Analysis of Variance (ANOVA) and Normalized Root Mean Square Error (NRMSE). The results revealed that the extraction efficiency was highly dependent on HNO_3 concentration, whereas the substituting effect of H_2O_2 was limited and occasionally acted as an extraction-inhibitory factor (e.g., for Fe). Through multi-objective optimization, a unified optimal condition (47.4% HNO_3 and 5.8% H_2O_2) was derived, demonstrating the capability to extract approximately 94% of multiple minerals for a wide range of P-F-C matrices.

1. Introduction

Minerals are abundantly found in the environment, such as in soil and water, and are essential for maintaining human health. However, minerals cannot be synthesized within the body and must be ingested in some form; an excess or deficiency of minerals can lead to various diseases. Therefore, knowledge regarding the amount of minerals ingested from food is nutritionally important, and precise quantitative analysis of minerals in food is indispensable for accurate intake assessment.

To measure minerals in food, organic matter such as proteins, fats, and carbohydrates (P-F-C) must first be removed. This is necessary to release the minerals retained in the organic matter and to break physical structures that interfere with the measurement. Common methods for removing organic matter include dry ashing, which literally turns the sample to ash by strong heating, and wet ashing, which involves strong heating in an acidic environment such as nitric acid (HNO_3). Furthermore, other methods have also been investigated, and studies on ultrasound-assisted extraction and the ratio of acids used have been reported in recent years (Acar et al. 2016; Ashoka et al. 2009; Krishna and Arunachalam 2004; Lindenmayer et al. 2023).

In particular, the application of microwave-assisted digestion to various foods is being explored, notably by the AOAC International (AOAC International 2005, 2011a, 2011b; Farias et al. 2022; Grossbier and Schoenfuss 2021; Jorhem and Engman 2000; Yutani et al. 2016). This method is effective for measuring highly volatile minerals and is becoming more widely used from a safety perspective, as it simplifies the management of harmful nitrogen oxides (NO_x) generated during digestion. Moreover, from the perspective of green chemistry, methods to reduce HNO_3 usage by substituting it with hydrogen peroxide (H_2O_2) have also been investigated (da Silva et al. 2020; Lee et al. 2022; Lo Dico et al. 2018; Santos et al. 2019). However, most of these studies have been verified against single food matrices and focus

primarily on toxic heavy metals. Optimization for minerals generally known as nutritional components and the investigation of digestion conditions that can be universally used for a wide range of food matrices are limited.

Moreover, advances in simultaneous analysis using inductively coupled plasma mass spectrometry (ICP-MS) have enabled the concurrent quantification of numerous minerals. However, because minerals possess diverse physical properties and chemical forms, their optimal extraction conditions vary; simply utilizing high concentrations of acid is not necessarily optimal for all minerals. In actual food analysis settings, mineral extraction is often performed using excessive amounts of HNO_3 or H_2O_2 , unnecessarily high temperatures, and long processing times. Therefore, we explored the optimal conditions for HNO_3 and H_2O_2 in microwave-assisted digestion applicable to a wide range of food matrices and minerals using a quadratic polynomial model.

In this study, target foods were selected based on general foods and their P-F-C balance. Three types of foods with numerous reports on mineral analysis (Abd EL Rahim et al. 2012; Bakircioglu et al. 2011; de Oliveira Filho et al. 2022; Lo Dico et al. 2018; Mohamed et al. 2020)—chocolate (F:C $\approx$ 1:1), cheese (P:F $\approx$ 1:1), and nori (P:C $\approx$ 1:1)—were chosen as representatives of food matrices. In addition, a multivitamin/multielement tablet (vitamin tablet) was added as a reference material. The measurement targets included 11 minerals related to nutritional labeling in Japan (Na, Mg, K, Ca, Cr, Mn, Fe, Cu, Zn, Se, and Mo, excluding P and I), Co as a trace mineral, and Pb, which is widely monitored as a food contaminant.

2. Materials and Methods

2.1. Reagents and samples

Nitric acid (for electronic industry, assay 70.3 %), hydrogen peroxide (for atomic absorption analysis, assay 35.4 %), and hydrochloric acid (for toxic metal measurement, assay 36.1 %) used for the analysis were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). A multi-mineral standard solution (Supelco Multi-element Standard Solution 1 for ICP, Sigma-Aldrich, St. Louis, MO, USA) and a selenium standard solution (Se Standard Solution (1000), FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) were used as standard solutions for preparing calibration curves. Sc, Y (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), and Ge (Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 1000 mg/L were used as internal standard substances. Ultrapure water purified by an ultrapure water system (Milli-Q IQ 7005, Merck Millipore, Darmstadt, Germany) was used for diluting and making up the volume of each sample. A 1 % HNO_3 solution containing 50 $\mu\text{g/L}$ of each internal standard substance (IS/ HNO_3) was used for sample dilution.

A vitamin tablet (NIST 3280 SRM Multivitamin/multielement tablets, NIST, Gaithersburg, MD, USA) was used to evaluate inorganic mineral extraction behavior. Food samples with a P:F, F:C, or C:P balance of approximately 1:1 were selected based on the Standard Tables of Food Composition in Japan; specifically, chocolate, cheese, and nori (dried seaweed) were chosen. Additionally, cheese was freeze-

dried before being used as a sample to eliminate the dilution effect of moisture. Based on the nutritional labeling of the purchased products, the protein:fat:carbohydrate contents of the chocolate, cheese (after freeze-drying), and nori were 10.0, 40.0, 44.0 g/100 g; 45.4, 43.9, 2.3 g/100 g; and 44.0, 4.0, 47.0 g/100 g, respectively.

2.2. Instruments and measurement conditions

A Multiwave 5000 (Anton Paar, Graz, Austria) was used as the microwave digestion system, utilizing a 20-position rotor and corresponding dedicated vessels. An inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7850, Agilent Technologies, Santa Clara, CA, USA) was used for mineral quantification. To reduce polyatomic ion interferences derived from the matrix, measurements were performed applying helium (He) mode and hydrogen (H₂) mode depending on the target element (Table 1, 3). MassHunter 5.2 (Agilent Technologies, Santa Clara, CA, USA) was used for instrument control and data acquisition.

2.3. Sample digestion conditions

A 0.5 g of the sample was accurately weighed into a vessel, and ultrapure water was added in advance so that the final reaction volume would be 5 mL. HNO₃ (final concentrations: 8.4, 32.3, 56.2 %) and H₂O₂ (final concentrations: 0.7, 3.9, 7.1 %) were added under three-level concentration conditions, respectively, followed by wet digestion using a microwave digestion system in a closed vessel (Table 2). The microwave digestion involved heating to 220 °C over 25 minutes and holding at 220 °C for 10 minutes. Afterwards, the vessels were cooled to 70 °C, removed from the microwave system, and cooled to room temperature. After digestion, 1 mL of hydrochloric acid was added to each decomposition vessel, and the volume was made up to 50 mL with ultrapure water. Subsequently, the digested samples were filtered through a cellulose acetate filter (0.45 µm) as necessary, and solutions diluted 10, 100, or 1000 times with IS/HNO₃ according to the concentration of the target minerals were used as samples for ICP-MS measurement.

2.4. Mineral quantification

A specified amount of the Se standard solution was added to the multi-mineral standard solution, and standard solutions serially diluted using IS/HNO₃ were measured to prepare a calibration curve. The calibration curve was divided into four segments (Seg1-4) according to the concentration range, and each segment was constructed using three standard solutions. Calibration curves were constructed by plotting the ratio of the counts of each mineral to the counts of the internal standard substance. The internal standard closest in atomic weight to the target mineral was selected. Furthermore, to correct for contamination derived from reagents, a blank sample prepared by diluting each reagent used for digestion 10-fold with IS/HNO₃ was measured. The obtained background count/IS count ratio was

calculated, and background correction was performed by subtracting this value from the sample signal according to the amount of reagent actually used in the digestion process.

2.5 Statistical analysis

Response Surface Methodology (RSM) based on a 3-level, 2-factor full factorial design (3^2) was applied to optimize the mineral extraction conditions in this study. HNO_3 concentration (X_1) and H_2O_2 concentration (X_2) were selected as independent variables, and each factor was set at three levels (-1, 0, 1). A total of 9 experimental conditions were performed, including replicate experiments to evaluate the pure error. Using the obtained extraction concentration (Y) of each mineral as the response variable, the following quadratic polynomial model was constructed using ordinary least squares (OLS):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2$$

Here, β_0 is the intercept of the model, β_1 and β_2 are the linear coefficients (main effects), β_{11} and β_{22} are the quadratic coefficients (curvature effects), and β_{12} represents the interaction coefficient between the factors.

The statistical significance and validity of the constructed response surface models were evaluated using analysis of variance (ANOVA). The overall significance of the model was confirmed by the F-test ($p < 0.05$) and the Lack of Fit (LoF) test ($p > 0.05$). Furthermore, to relatively compare and evaluate the predictive accuracy of the models and the extraction robustness for matrices, the Normalized Root Mean Square Error (NRMSE, %) was calculated in addition to the adjusted coefficient of determination (Adj-R^2). For the Model p-value indicating the significance of the model, $p < 0.05$ was considered statistically significant (Good). For Adj-R^2 , which indicates the explanatory power of the model, values > 0.80 were defined as high explanatory power (Good), $0.60\text{--}0.80$ as acceptable, and < 0.60 as insufficient (Poor). The LoF test, which evaluates the structural fitness of the model, was assessed as having no lack of fit (Valid / Good) when $p > 0.05$. The NRMSE was calculated as the ratio of the root mean square error to the range of observed values (maximum minus minimum), and models with $\text{NRMSE} \leq 0.15$ were defined as having high predictive accuracy (Good fit), $0.15 < \text{NRMSE} \leq 0.20$ as practically acceptable (Acceptable fit), and > 0.20 as having poor predictive accuracy (Poor fit). Additionally, to visually identify the contribution of each factor (linear, quadratic, and interaction terms) to the extraction efficiency, Pareto charts were constructed based on the absolute values of the standardized effects (t -values), and their significance was evaluated against a reference line at $p = 0.05$.

Subsequently, 3D response surface plots were generated from the constructed models. Multi-objective optimization using the desirability function was employed to derive the optimal conditions according to the characteristics of each food matrix. During this process, models that were classified as 'poor' for all evaluation criteria in the model adequacy test were excluded from the optimization. All statistical procedures, including ANOVA and graphical plotting, were performed using Python (statsmodels, scipy, and matplotlib).

3. Results

3.1 Mineral analysis of vitamin tablets and foods

The vitamin tablet and three types of food were subjected to microwave-assisted digestion after adjusting the concentrations of HNO_3 and H_2O_2 to three levels each (Table 2). The results of the ICP-MS measurements are presented in Table 3. In the vitamin tablets, the mean values of 8 minerals (Na, Mg, K, Ca, Mn, Cu, Zn, and Pb) fell within the range of the NIST certified values $\pm$ expanded uncertainty. Among these minerals, those other than Cu and Pb had a relative standard deviation (RSD) $< 5\%$ for all measurement results of all other foods as well. In addition, Co in the vitamin tablets slightly deviated from the expanded uncertainty range but had an RSD $< 5\%$. Therefore, an analysis using quadratic polynomial response surface models was performed for the eight minerals (Cr, Fe, Co, Cu, Se, Mo, and Pb) whose extraction efficiencies depended on the conditions.

3.2 Model suitability evaluation

Quadratic polynomial response surface models with HNO_3 and H_2O_2 concentrations as independent variables were developed (Online Resource 1). The validity of these models was comprehensively evaluated using ANOVA, Adj-R^2 , the LoF test, and the NRMSE (Table 4). Overall, 24 out of the 28 models (4 samples $\times$ 7 minerals) were statistically significant (Model $p < 0.05$). In most models, the NRMSE met the criteria for "Good fit" ($< 15\%$) or "Acceptable" ($15\text{--}20\%$), while 4 models were evaluated as "Poor" across all evaluation axes. Evaluating the effect sizes using Pareto charts (Fig. 1), an increasing trend in mineral extraction was observed with increasing HNO_3 concentration for almost all minerals ($p < 0.05$). The influence of the quadratic term of HNO_3 ($\text{HNO}_3 \times \text{HNO}_3$) on Mo extraction was negative for all samples. Similar phenomena were observed for Cr and Fe in the vitamins and chocolate, and for Se in cheese.

3.3 Response surface plots and exploration of optimal conditions

In the vitamin tablets, Cr responded to the addition of H_2O_2 under low- HNO_3 conditions (Fig. 2). The extraction of Fe decreased under high- HNO_3 conditions. For Se, the response surface indicated a negative correlation with HNO_3 and a positive correlation with H_2O_2 . In chocolate, Pb extraction efficiency was maximized under conditions where both HNO_3 and H_2O_2 were highly concentrated. In cheese, the response surfaces showed no notable deviations from the ANOVA results. In nori, Fe extraction decreased under conditions of low HNO_3 and high H_2O_2 , whereas it reached 93 % of the maximum value under low- HNO_3 and low- H_2O_2 conditions. Se and Mo correlated positively with H_2O_2 concentration in low- HNO_3 environments. Multi-objective optimization using the desirability function (Table 5) was conducted to identify optimal conditions for simultaneous extraction, excluding models evaluated as "poor". These estimation results yielded an overall desirability score (geometric mean of predicted extraction rates) of 95.4 % or higher for all food samples. The optimal condition for the vitamin tablets was 38.7 % HNO_3 , yielding a minimum estimated extraction rate of 97.1 % for Pb. The optimal HNO_3 concentration for chocolate and nori was 56.2 %. Under these conditions, the extraction rates of Fe were the lowest in chocolate and nori (93.3 % and 84.4 %, respectively). In cheese, the optimal HNO_3 concentration was 51.0 %, yielding a minimum estimated extraction rate of 91.6 % for Pb. The optimal

H₂O₂ concentration was 4.1 % for vitamin tablet, 7.1 % for chocolate, 0.7 % for cheese, and 1.3 % for nori. A unified optimal condition using all data was estimated at 47.4 % HNO₃ and 5.8 % H₂O₂, yielding a geometric mean of 93.5 % with a minimum extraction of 77.8 % for Cr in nori.

4. Discussion

As the results showed that the mean values of eight minerals (Na, Mg, K, Ca, Mn, Cu, Zn, and Pb) in the vitamin tablets consistently fell within the NIST certified ranges across all investigated conditions (Table 3), it was confirmed that values close to the true concentrations were accurately obtained. Furthermore, the extraction amounts of Na, Mg, K, Ca, Mn, and Zn were unaffected by changes in extraction conditions across all examined food matrices. This observation is supported by the flat response surface plots (Online Resource 3) and the comparative dry ashing results (Online Resource 2). Additionally, shaking extraction with dilute hydrochloric acid is a common method for Na and K, indicating that complete digestion of the matrix with highly concentrated acid is not always necessary for these elements. Given that an analytical uncertainty of several percent is inherent to simultaneous multi-mineral analysis by ICP-MS (Ghuniem et al. 2018), the observed variations for these minerals fall within this margin of error, and they were therefore excluded from subsequent statistical analyses. Additionally, the high temperature of 220°C applied in this study indicates that even heavy metals like Cu can be dissolved with a small amount of acid if no organic bonds are present. Conversely, this suggests that extracting Cu from food matrices requires sufficient digestion of the organic matrix.

Regarding the statistical model suitability, the NRMSE meeting the criteria for "Good fit" or "Acceptable" for most models indicates that modeling by OLS was appropriate for optimizing extraction conditions. Crucially, the Pareto charts (Fig. 1) revealed the overarching main effects on extraction behaviors. The significant positive main effect of HNO₃ concentration was dominant for almost all minerals (Fig. 1). However, the negative influence of the HNO₃ quadratic term observed for Mo, as well as for Cr, Fe, and Se in specific matrices (Fig. 1), indicates that excessive HNO₃ either reduced extraction efficiency or reached a plateau. Furthermore, increasing the H₂O₂ concentration did not yield a consistent overall effect across elements (Fig. 1).

The characteristic 3D response surfaces (Fig. 2) provided further mechanistic insights into element- and matrix-specific interactions. In the vitamin tablets (Fig. 2a), although Cr responded to H₂O₂ under low-HNO₃ conditions, H₂O₂ could not completely substitute for HNO₃. The clear decrease in Fe extraction under high-HNO₃ conditions (Fig. 2a) was considered a specific phenomenon in samples with high Fe concentrations. In chocolate (Fig. 2b), the distinct behavior of Pb maximizing under highly concentrated HNO₃ and H₂O₂ differs from other foods, although it remains unclear whether this originates from chocolate's complex matrix or the chemical form of Pb. In nori (Fig. 2d), the unique decrease of Fe under low HNO₃ and high H₂O₂ was not observed in other foods. Conversely, the behavior of Se and Mo in low-HNO₃ environments (Fig. 2d) suggests that adding H₂O₂ has a specific extraction-promoting effect in those regions. Thus, the response surfaces clearly demonstrate that optimal extraction conditions occasionally conflicted depending on the mineral, even within the same sample.

Although the optimal HNO_3 concentration for chocolate and nori reached the maximum value (56.2%) within the scope of this study, the extraction rate was considered to be near its maximum when compared with the results of dry ashing (Online Resource 2). Considering this in conjunction with the results for the vitamin tablets, it was suggested that applying uniformly high concentrations of HNO_3 may inadvertently cause a decrease in the extraction rate of Fe. The varying requirements for H_2O_2 suggest it may be effective for mineral extraction in lipid- and carbohydrate-rich matrices like chocolate, but it introduces a trade-off by acting as an inhibitory factor for certain minerals, functioning as an adjusting factor in optimizing multi-mineral simultaneous analysis. Furthermore, applying multi-objective optimization (Table 5) demonstrated that identifying conditions to extract all target minerals simultaneously requires a compromise. Under these optimized conditions, certain elements effectively act as bottlenecks for the overall extraction efficiency, such as Pb in the vitamin tablets, Fe in chocolate and nori, and Cr when applying the unified condition across all data. Although several studies have reported that H_2O_2 reduces the residual carbon content (Barbosa et al. 2015; da Silva et al. 2020), this decrease does not necessarily translate to improved mineral extraction efficiency or ICP-MS measurements. Provided that the sample is adequately diluted, the dissolved carbon content in the extract might not be a critical issue.

These findings support the established consensus that performing simultaneous multi-mineral analysis requires fine-tuning extraction conditions based on the specific food matrix and element reactivity, rather than applying a standardized acid extraction method. Moreover, the effect of the P-F-C balance on mineral extraction was not clearly established within the examined ranges, suggesting that inherent mineral characteristics—such as solubility, concentration, and integration into molecular structures with covalent bonds—may have a greater influence on extraction efficiency than the P-F-C matrices themselves. However, recognizing that individual matrix-specific optimization is not always feasible in routine food analysis, this study explored a practical compromise. Consequently, we demonstrated that if an average recovery rate of approximately 94% is deemed acceptable for the analytical purpose, the unified condition estimated here (47.4% HNO_3 and 5.8% H_2O_2) can serve as a reasonable baseline to extract various minerals across a wide range of P-F-C matrices. While this condition leaves room for further methodological refinement, it provides a useful reference point for minimizing constant method readjustments.

5. Conclusions

In this study, we systematically evaluated microwave-assisted digestion parameters across diverse P-F-C matrices. As anticipated from conventional analytical knowledge, the extraction efficiencies of Na, Mg, K, Ca, Mn, and Zn were unaffected by the acid concentrations within the investigated range, confirming that highly concentrated acid digestion is unnecessary for these easily extractable elements.

Conversely, for elements whose extraction efficiencies varied depending on the conditions (Cr, Fe, Co, Cu, Se, Mo, and Pb), our results confirmed that while HNO_3 concentration significantly affects extraction, simply applying maximum acid concentrations can be counterproductive, as observed with Fe.

Furthermore, the ability of H₂O₂ to substitute for HNO₃ was found to be limited, functioning mainly as an adjusting factor with inherent trade-offs. These results highlight the known principle that fine-tuning the HNO₃ concentration relative to the specific matrix remains essential for highly accurate analyses.

Nevertheless, a potentially useful contribution of this study is the derivation of a unified condition. We estimated a unified condition of 47.4% HNO₃ and 5.8% H₂O₂ from all compiled data. This suggests that if a recovery rate of approximately 94% is acceptable, this single condition provides a practical compromise for extracting multiple minerals from diverse P-F-C matrices, even though further methodological improvements are possible.

Declarations

Competing Interests

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by I.S.. The first draft of the manuscript was written by I. S. and J. T. commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability Statements

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Abd EL Rahim A, Mohamed T, Tammam A (2012) Assessment of toxic heavy metal residues in some types of cheese by using ICP-OES. J Food Dairy Sci 3:725–733. <https://doi.org/10.21608/jfds.2012.81743>
2. Acar O, Tunçeli A, Türker AR (2016) Comparison of wet and microwave digestion methods for the determination of copper, iron and zinc in some food samples by FAAS. Food Anal Methods 9:3201–3208. <https://doi.org/10.1007/s12161-016-0516-4>
3. Alhagri I, Albeshry M (2023) Microwave-assisted digestion using dilute nitric acid and hydrogen peroxide for multielement determination in wheat flour by ICP-MS. J Chem:1–10.

<https://doi.org/10.1155/2023/2349838>

4. AOAC International (2005) Lead, cadmium, zinc, copper, and iron in foods: Atomic absorption spectrophotometry after microwave digestion (AOAC Official Method 999.10). In: Official methods of analysis of AOAC International. AOAC International
5. AOAC International (2011a) Calcium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium, and zinc in fortified food products: Microwave digestion and inductively coupled plasma-optical emission spectrometry (AOAC Official Method 2011.14). In: Official methods of analysis of AOAC International. AOAC International
6. AOAC International (2011b) Chromium, selenium, and molybdenum in infant formula and adult nutritional products: Inductively coupled plasma-mass spectrometry (AOAC Official Method 2011.19). In: Official methods of analysis of AOAC International. AOAC International
7. Ashoka S, Peake BM, Bremner G, Hageman KJ, Reid MR (2009) Comparison of digestion methods for ICP-MS determination of trace elements in fish tissues. *Anal Chim Acta* 653:191–199. <https://doi.org/10.1016/j.aca.2009.09.025>
8. Bakircioglu D, Kurtulus YB, Ucar G (2011) Determination of some traces metal levels in cheese samples packaged in plastic and tin containers by ICP-OES after dry, wet and microwave digestion. *Food Chem Toxicol* 49:202–207. <https://doi.org/10.1016/j.fct.2010.10.017>
9. Barbosa JTP, Santos CMM, Peralva VN, Flores EMM, Korn M, Nóbrega JA, Korn MGA (2015) Microwave-assisted diluted acid digestion for trace elements analysis of edible soybean products. *Food Chem* 175:212–217. <https://doi.org/10.1016/j.foodchem.2014.11.092>
10. da Silva IJS, Lavorante AF, Paim APS, da Silva MJ (2020) Microwave-assisted digestion employing diluted nitric acid for mineral determination in rice by ICP OES. *Food Chem* 319. <https://doi.org/10.1016/j.foodchem.2020.126435>
11. de Oliveira Filho EF, Miranda M, Ferreiro T, Herrero-Latorre C, Castro Soares P, López-Alonso M (2022) Concentrations of essential trace and toxic elements associated with production and manufacturing processes in Galician cheese. *Molecules* 27:4938. <https://doi.org/10.3390/MOLECULES27154938>
12. Farias AS, Santos HM, da Silva Junior ALS, da Silva VHC, Mendonça RBES, Coutinho JP, Lôbo IP, de Jesus RM (2022) Multivariate approaches applied to optimization of an ultrasound-assisted extraction procedure for determination of essential elements in guarana samples by ICP OES. *Food Sci Technol* 42:e01321. <https://doi.org/10.1590/fst.01321>
13. Ghuniem MM, Korshed MA, Souaya ER (2018) Optimization and validation of an analytical method for the determination of some trace and toxic elements in canned fruit juices using quadrupole inductively coupled plasma mass spectrometer. *J AOAC Int.* <https://doi.org/10.5740/jaoacint.18-0022>
14. Grossbier DT, Schoenfuss TC (2021) Using microwave-accelerated digestion instead of dry ashing during sodium analysis of low-moisture, part-skim mozzarella. *JDS Commun* 2:13–15. <https://doi.org/10.3168/jdsc.2020-0008>

15. Jorhem L, Engman J (2000) Determination of lead, cadmium, zinc, copper, and iron in foods by atomic absorption spectrometry after microwave digestion: NMKL collaborative study. *J AOAC Int* 83:1189–1203.
16. Krishna MVB, Arunachalam J (2004) Ultrasound-assisted extraction procedure for the fast estimation of major, minor and trace elements in lichen and mussel samples by ICP-MS and ICP-AES. *Anal Chim Acta* 522:179–187. <https://doi.org/10.1016/j.aca.2004.07.006>
17. Lee J, Park YS, Lee HJ, Koo YE (2022) Microwave-assisted digestion method using diluted nitric acid and hydrogen peroxide for the determination of major and minor elements in milk samples by ICP-OES and ICP-MS. *Food Chem* 373. <https://doi.org/10.1016/j.foodchem.2021.131483>
18. Lindenmayer R, Lu L, Eivazi F, Afrasiabi Z (2023) Atomic spectroscopy-based analysis of heavy metals in seaweed species. *Appl Sci* 13:4764. <https://doi.org/10.3390/APP13084764>
19. Lo Dico GM, Galvano F, Dugo G, D'ascenzi C, Macaluso A, Vella A, Giangrosso G, Cammilleri G, Ferrantelli V (2018) Toxic metal levels in cocoa powder and chocolate by ICP-MS method after microwave-assisted digestion. *Food Chem* 245:1163–1168. <https://doi.org/10.1016/j.foodchem.2017.11.052>
20. López AR, Ortega-Caneda E, Espada-Bellido E, Taracena-Zepeda OR, Palma M, Fernández-Barbero G (2024) Quantification of minerals in edible mushrooms via optimized microwave-assisted digestion: Nutritional contributions of Fe, Mg, Na, K, and Ca. *Foods* 13:4051. <https://doi.org/10.3390/foods13244051>
21. Mohamed R, Zainudin BH, Yaakob AS (2020) Method validation and determination of heavy metals in cocoa beans and cocoa products by microwave assisted digestion technique with inductively coupled plasma mass spectrometry. *Food Chem* 303. <https://doi.org/10.1016/j.foodchem.2019.125392>
22. Santos HM, Coutinho JP, Amorim FAC, Lôbo IP, Moreira LS, Nascimento MM, de Jesus RM (2019) Microwave-assisted digestion using diluted HNO₃ and H₂O₂ for macro and microelements determination in guarana samples by ICP OES. *Food Chem* 273:159–165. <https://doi.org/10.1016/j.foodchem.2017.12.074>
23. Yutani A, Kishi E, Ozaki A, Shinya M, Ooshima T, Yamano T (2016) Development and validation of simultaneous analysis of minerals and toxic elements in foods by ICP-MS. *Food Hyg Saf Sci* 57:57–65. <https://doi.org/10.3358/shokueishi.57.57>

Tables

Tables 1 to 5 are available in the Supplementary Files section.

Figures

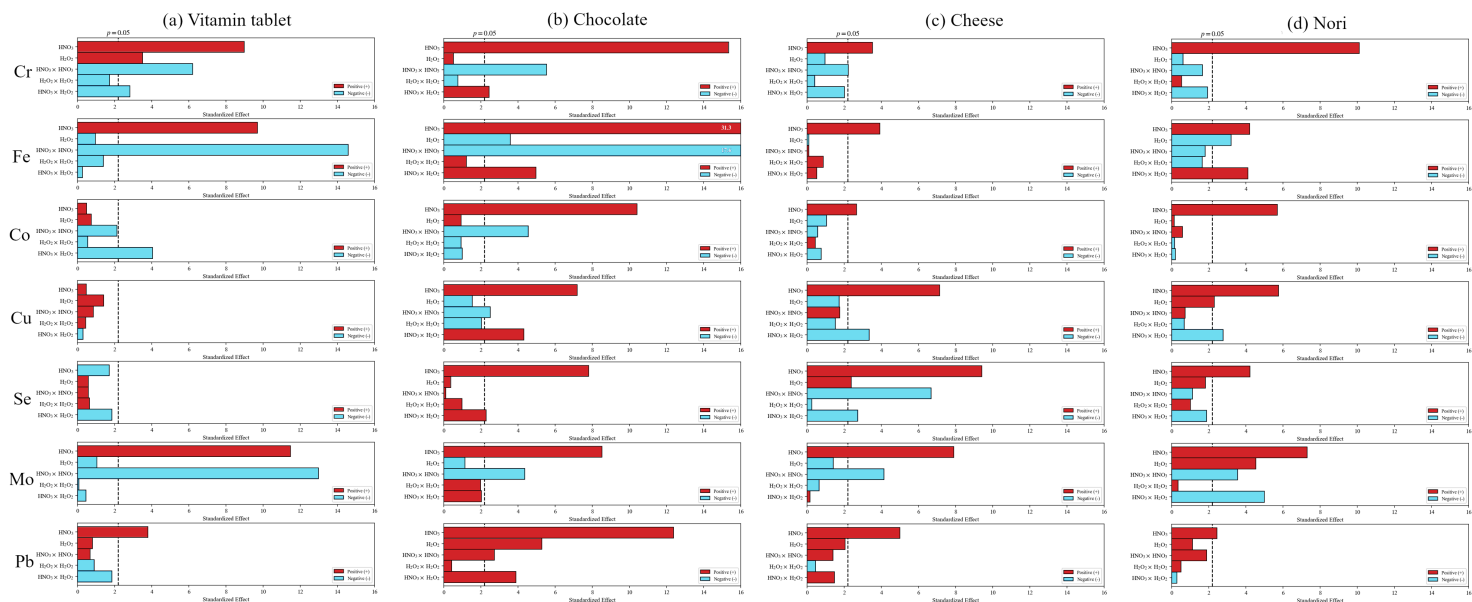


Figure 1

Standardized main and interaction effects of HNO_3 and H_2O_2 concentrations on the extraction efficiency of each mineral.

Pareto charts illustrating the standardized effects of HNO_3 , H_2O_2 , and their interactions on the extraction of each mineral. The vertical dotted line indicates statistical significance at $p = 0.05$.

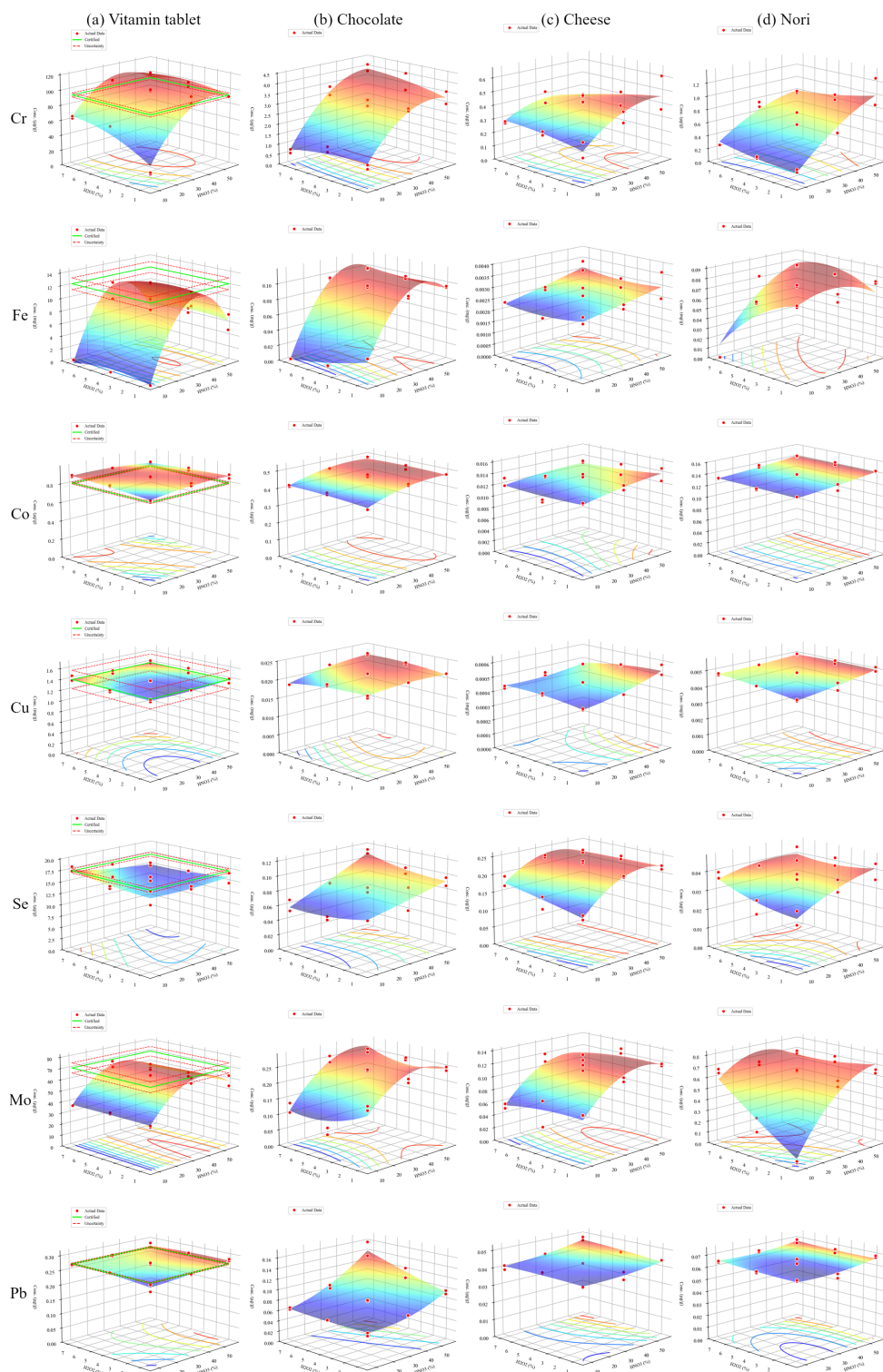


Figure 2

3D response surface plots showing the effects of HNO_3 and H_2O_2 concentrations on mineral extraction efficiencies

Panels correspond to (a) vitamin tablet, (b) chocolate, (c) cheese, and (d) nori for representative minerals (Cr, Fe, Co, Cu, Se, Mo, and Pb). The interactive surfaces are generated from the quadratic polynomial

equations optimized via Response Surface Methodology (RSM). Red points represent the observed experimental values.

Supplementary Files

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