



Critical evaluation of energy dispersive X-ray fluorescence spectrometry for multielemental analysis of coffee samples: Sample preparation, quantification and chemometric approaches

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ARTICLE INFO

Keywords:

EDXRF
Coffee
PCA
PLS-DA
Multielemental analysis

ABSTRACT

In the present contribution, a critical evaluation of the use of EDXRF (Energy dispersive X-ray fluorescence spectrometry) for elemental composition determination (K, Ca, Mn, Fe, Cu, Zn, Sr and Rb) of coffee samples is presented. Firstly, several sample treatment procedures (pressed pellets and loose powder) and quantification strategies (fundamental parameters and empirical calibration) were evaluated.

In addition to quality and nutritional purposes, information of elemental content in coffee samples can be also useful for coffee traceability and provenance studies. In view of that, a comparison of different tools such as explorative principal component analysis (PCA) and classification by partial least square regression (PLS-DA) were also tested as discriminating tools of different types of coffee samples (i.e., pure, instant and coffee with additives). Chemometric analysis was performed using coffee elemental concentrations determined by the developed EDXRF method and, for the first time, using raw EDXRF spectra obtained, without the need of a fitting and quantification procedure.

1. Introduction

Coffee is one of the most consumed beverages in the world. As a figure, the International Coffee Organization (ICO) estimated the worldwide coffee consumption to be 165.4 million bags in the year of 2020/21 [1].

Although the content of elements (others than those of the coffee organic matrix) in coffee is only about 5%, their determination is important since, depending on the concentration and type, they can affect the quality of the product [2]. Some microelements present in coffee are biochemically important from a health point of view. For instance, Fe, Zn, Cu and Mn have recently demonstrated antioxidant properties [3]. On the contrary, other potential toxic elements (i.e., Cd, Pb, Hg) can also be found in coffee grains depending on the planting location, fertilizers and pesticides or contamination during the grinding or storage process [4]. In view of that, the determination of elemental composition of coffee samples is of significance for quality, safety and

nutritional purposes.

Usually, spectroscopic techniques such as inductively coupled plasma-mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP-OES) and flame atomic absorption spectrometry (FAAS) have been used for elemental analysis of powdered coffee samples. However, the use of these techniques requires sample preparation procedures for total destruction of the organic matrix by chemical treatments [5]. Nowadays, there is an increasing demand for methods that implicate less or no sample pretreatment for multi-elemental analysis of foodstuff samples, which are more in line with the principles of Green Analytical Chemistry [6]. Most X-ray fluorescence spectrometry (XRF) techniques are adequate for this purpose since they present the possibility to perform almost direct and multi-element analysis of solid samples, qualitative, semiquantitative and quantitative determination in a wide dynamic range and are really cost effective [7]. Additionally, the non-destructive nature of XRF methods enables re-measurements of the same sample, which can be of significance for

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<https://doi.org/10.1016/j.sab.2024.106898>

Received 3 January 2024; Received in revised form 10 March 2024; Accepted 12 March 2024

Available online 15 March 2024

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quality control purposes.

In Table S1, an overview of the XRF methods published in the last years for multielemental analysis of powdered coffee samples is displayed [2,8–14]. Usually, energy dispersive XRF systems (EDXRF) are preferred for this purpose due to the possibility to get simultaneous multielemental information. As it is shown, there is a lack of consensus about the best way to prepare coffee samples as well as the quantification approach used. In most contributions, coffee samples are prepared pressing an amount of powder (0.2–5 g) into a pellet ($\varnothing = 13\text{--}40$ mm) before XRF measurements. In a few contributions (all of them dealing with the use of portable XRF systems) coffee samples are directly measured by pouring an amount of coffee powder in a cuvette (loose powder). There are also different quantification options used in multielemental analysis of coffee samples by XRF. In view of the lack of commercially available coffee reference materials, the most commonly used quantification strategies are based on standard-less fundamental parameters (FP) procedures. Only in two scientific articles an external calibration using a set of reference materials (with a mixture of inorganic and organic matrices) or a set of coffee samples (previously characterized using a reference technique) is used [2,8]. Practical applications of XRF for coffee analysis can also be found in a dedicated chapter in the recent published book “X-ray fluorescence in biological sciences: principles, instrumentation and applications” [15].

In addition to safety and nutritional purposes, information on elemental content in coffee samples can also be useful for coffee traceability and provenance studies [14]. This approach is also of particular relevance to the area of product authentication in order to establish the origin or processing procedures of coffee. So far, several research attempts have been carried out to find reliable methods that can correctly differentiate product provenance. Most of these studies are based on the combined use of elemental composition measurements with chemometric tools [16]. As it is shown in Table S1, exploratory, classification and discrimination approaches such as principal component analysis (PCA), cluster analysis partial least square discriminant analysis (PLS-DA) and linear discriminant analysis (LDA) have been used for traceability and provenance studies of coffee samples when using XRF as an analytical technique. In all cases, elemental concentrations have been used as variables to carry out the chemometric analysis.

However, unlike other atomic spectroscopic techniques used to determine the elemental composition of coffee samples (i.e., ICP-MS, ICP-OES, FAAS), EDXRF provides a continuous spectrum as an output, which could be used as a sample fingerprint. This approach has been widely used in molecular analysis such as near infrared spectroscopy (NIRS) [17], but has been little explored in combination with EDXRF spectrometry. Based on the authors' knowledge, very few contributions exist on this topic and all of them are dealing with other research fields including the determination of soil organic carbon determination (Santos et al. [18]) or for the identification of falsified medical products [19]. Only, in very few recent contributions the feasibility of using XRF spectra in combination with multivariate analysis in the food field have been proposed, including the classification of beans seeds [20], tomatoes and sweet paper [21] according to their geographical origin. In the present work, for the first time, the use of raw EDXRF spectra in combination with PCA and PLS-DA has been tested as a simple and fast discriminating tool of different types of coffee samples (pure, instant and with additives). For comparison purposes, and in order to demonstrate the real capability of such a method, elemental concentrations (K, Ca, Mn, Fe, Cu, Zn, Rb and Sr) determined by EDXRF have also been used as variables in chemometric analysis. In this sense, a carefully study of the best experimental conditions to obtain reliable element concentrations in coffee samples by EDXRF has also been carried out. On the one hand, different samples treatment procedures including the preparation of a pressed pellet of the coffee powder (with and without the addition of a binder) or the analysis of the coffee as a loose powder have been evaluated. On the other hand, two different quantification approaches have been also tested: (i) a FP method using a sample matrix composition

determined experimentally from the ratio of measured scatter peak intensities and (ii) an external calibration using a set of coffee samples previously characterized by ICP-OES.

2. Experimental

2.1. Reagents and materials

Stock solutions of $1000\text{ mg}\cdot\text{L}^{-1}$ (ROMIL PrimAg® Monocomponent reference solutions) were used to prepare calibration standards for ICP-OES analysis. Ultrapure deionized water (Millipore Corp., Bedford, Massachusetts, USA) was used to dilute the aforementioned stock solutions and coffee sample digests. Nitric acid (69%, HIPERPUR, Panreac, Spain) and hydrogen peroxide solution (30%, TraceSELECT®, Sigma-Aldrich, Spain) were employed to digest coffee samples. To stabilize the pellets made from coffee powder Hoechst wax C micropowder ($\text{C}_{38}\text{H}_{76}\text{N}_2\text{O}_2$, Merck KGaA, Germany) was used.

Different certified reference materials (CRMs) with an organic matrix were employed to verify the quality of the results obtained by ICP-OES analysis: GBW 07604 (Poplar leaves), NCS ZC73036 (Green Tea) and NCS ZC73031 (Carrot) purchased from China National Analysis Center for Iron and Steel (Beijing, China).

2.2. Coffee samples

Coffee samples were purchased from Croatian markets. Different types of varieties were selected including pure, instant and coffee with additives (i.e., with milk, chocolate, vanilla, Irish cream and hazelnut). In all cases, samples were presented in bags of 2 g of powder material.

A total number of 48 coffee samples were considered (19 pure coffees, 8 instant coffees and 24 coffees with additives). A set of 11 of these samples was used as calibration standards for EDXRF analysis, when using empirical calibration as the quantitative method.

2.3. Multielemental analysis of coffee samples

2.3.1. Energy dispersive X-ray fluorescence spectrometry (EDXRF) method

Coffee samples were prepared as pressed pellets of 40 mm in diameter by mixing 2 g of powder sample with 0.2 g of WAX and pressing the mixture at 20 mt for 60 s. This procedure was employed for all types of coffee samples tested (pure, instant and with additives). For comparison purposes, coffee samples were also analyzed by EDXRF as loose powders. For that, 2 g of coffee were poured into a special Teflon cup which incorporates a snap-on ring at the end of the cell for attachments of thin-film supports. In this case, a $4.0\text{ }\mu\text{m}$ -thick Prolene® X-ray film (supplied by Chemplex Industries, Inc., Palm City, FL, USA) was used.

A commercially available benchtop EDXRF spectrometer (S2 Ranger, Bruker AXS, GmbH, Karlsruhe, Germany) was employed for coffee analysis. This instrument is equipped with a Pd target X-ray tube (max. power 50 W) and an XFLASH LE Silicon Drift Detector (SDD) with an ultrathin beryllium window ($0.3\text{ }\mu\text{m}$ thickness) and a resolution less than 129 eV at the Mn K α line for a count rate of 100,000 counts per second (counts/s). The instrument is also equipped with nine primary filters and it can operate under vacuum conditions or He atmosphere. An additional advantage of the EDXRF system used includes low operating costs because it does not require any cooling media to function. The software used to control the equipment and to perform the data treatment was Spectra EDX (Bruker AXS, GmbH, Germany). This software can perform full line profile fitting, deconvolution when lines are overlapped, intensity correction for interelement effects and full-quantitative routines. Specific measurement conditions used for the analysis of coffee samples are displayed in Table S2.

2.3.2. Inductively coupled plasma emission spectroscopy (ICP-OES) method

In view of the lack of coffee reference materials, a set of coffee samples was also analyzed by ICP-OES and the determined elemental

concentrations were used as reference values.

A microwave acid digestion method was employed for the digestion of coffee samples prior to the ICP-OES analysis. In a previous publication we already demonstrated the suitability of this method for the complete digestion of organic matrix samples (vegetation) [22]. In brief, about 250 mg of sample was added in high-density Teflon vessels with 9 mL of nitric acid and 1 mL of hydrogen peroxide. The vessels were closed and heated following a two-stage digestion program consisting of a first step of 5 min to reach 180 °C and a second step of 10 min at 180 °C. After cooling, digested sample solutions were transferred to a 30 mL flask and brought to volume with ultrapure water. For measurements, an Agilent ICP-OES 5100 Synchronous Vertical Dual View (SVDV) spectrometer was used. Specific instrument characteristics and measurement conditions used are displayed in Table S2.

To verify the good quality of the results obtained by the ICP-OES method three organic reference materials were analyzed. As it is shown in Table S3, in most cases good agreement was assessed between concentrations obtained by the ICP-OES method and reference values. This fact, reinforces the suitability of using element concentrations determined by the ICP-OES method as reference values.

2.4. Chemometric analysis

Chemometric analysis was carried out using the IBM SPSS program [Version 28]. Principal Component Analysis (PCA) and Partial Least Squares combined with Discriminant Analysis (PLS-DA) techniques were considered to analyze the data. As for the spectra treatment, a simple in-house made Fortran 95 program [Lahey/Fujitsu Fortran 95 express v 7.20.00 compiler] allowed the reading of the .txt files provided by the spectrometer and the subsequent data alignment. Another in-house program was used to automate the cross-validation leave-one-out (LOO) procedure described below. The program codifies the same equations and algorithms as SPSS, but with the difference that the calculation procedure is automated. This feature enabled us to perform the entire LOO, obtaining, as it was verified afterwards, the same results as the commercial program. As the number of available samples is small and also there are several groups defined, the LOO procedure was selected as the cross-validation technique.

2.4.1. Principal component analysis (PCA)

PCA is a widespread technique used to reduce the data dimensionality. After data standardization, the procedure consists of the application of a change of basis. The new basis vectors are linear combinations of the original sample descriptors and are automatically selected in such a manner that the new axes are orthogonal and collecting in their projections the biggest amount possible of data variability. Mathematically, those conditions lead to a simple procedure: the data matrix is symmetrized conforming a correlation matrix and the resulting eigenvectors are the new descriptors or orthogonalized principal components (PC). The contributions of each original variable in each principal component are called loadings, whereas the new coordinates of the samples in terms of the new axes are called scores. Additionally, it is demonstrated that the eigenvalues attached to each eigenvector indicates the proportion of data variance retained in it. In this manner, the principal components are sorted from bigger eigenvalues to lesser ones. Usually, only the first two or three eigenvectors are considered in the new sample Cartesian representations. It is noteworthy that a PCA calculation does not involve the use of the samples property dependent value or category. The property value is depicted after the new sample scores are obtained and represented. It is expected that the new representation would be able to unveil appropriate groupings or associations of samples.

2.4.2. Partial least squares regression (PLS)

In some sense, PLS constitutes a related procedure to PCA. The main difference is found in the fact that PLS takes into account the samples' property values or category in the calculations. The algorithm combines

iterative steps related to diagonalization and regression [23]. The final result is once again a set of orthogonal axes, here called Latent Variables (LV), which can be interpreted in the same way as PCs. However, this time they are simultaneously optimized to correlate with the samples' property values or categories. The algorithmic procedure also provides with discriminating functions, as it will be explained below. In particular, if the samples are classified into three classes, the PLS-DA model consists of two discriminating functions. The representations of the samples according to the two functions, acting as new axes, should reveal a group separation in a bidimensional map. Usually, the discriminant function values attached to each sample can be interpreted as being the probabilities (despite small negative values or values slightly greater than the unit can be obtained) of belonging to a certain group.

3. Results and discussion

3.1. Evaluation of coffee sample preparation for EDXRF analysis

As shown in Table S1, there is no standardized procedure to prepare coffee powder samples for XRF analysis. In most of the studies, coffee samples are prepared as pressed pellets of the powdered material but even in this case, different procedures are used to obtain the pellets (using only the raw material, addition of a binder, etc.). In this study, different procedures to prepare coffee samples were tested. In all cases, the amount of sample used was fixed to 2 g, since many of the coffee samples under investigation were packed in boxes containing this amount. In a first set of experiments, the best way to prepare pressed pellets was studied. Considering the morphology of coffee, pellets were prepared using the raw powdered material but also after the addition of 10% and 20% of wax as a binder. Wax is an organic compound widely used to hold sample particles together [24]. In Table S4, the effect of the wax content used to prepare coffee pellets on signal-to-noise ratio of analytes is shown. For all types of coffee (instant, pure and with additives), the signal-to-noise ratio decreases when the content of wax increases. This fact can be related to the dilution of the sample with the addition of the binder but also due to the matrix of the binder itself (composed of light elements), which can increase the background of the EDXRF spectrum. This effect, as it is shown in Table S4, is more relevant in the case of trace elements (i.e., Mn determination in coffee with additives). Finally, pressed pellets were prepared by adding 10% of wax as a compromise between an acceptable signal-to-noise ratio and obtaining a more resistant and stable coffee pellets over time.

In addition, an easier and faster sample preparation strategy was also tested for coffee sample analysis by EDXRF. It consisted of the direct analysis of the loose coffee powder by depositing 2 g of the material in a sample cup without any additional preparation (see section 2.3.1 for details). In Table S5 a comparison of the sensitivity and precision of the results obtained for the analysis of five replicates of a pure coffee sample using loose powder and pressed pellet preparation is displayed. As it is shown, in all cases, better results in terms of sensitivity and precision were obtained when preparing coffee samples as pressed pellets. Surely, the bulk density in loose powder preparation may not be consistent and that the grain size variation may cause the XRF readings to vary. In view of the obtained results, coffee samples were prepared as pressed pellets (2 g of coffee with 0.2 g of wax).

3.2. Measurement conditions for multielemental EDXRF analysis

To obtain the most efficient excitation for analyte determinations, an evaluation of the best EDXRF measuring conditions was carried out. Tests were performed by analyzing a pressed pellet of a pure coffee sample. The intensity was adjusted automatically to obtain a maximum count rate of 100,000 cps and the measuring time (200 s) was selected as a trade-off between an acceptable repeatability of measurements and total analysis time. In this study, the choice of the primary beam filters

and the tube operating conditions was based on the best signal-to-noise ratio attainable for each element. In Fig. 1, EDXRF spectra obtained for the different measurement conditions are shown. It was found that for light Z elements (K, Ca), the best choice was using a voltage of 20 kV without the use of any primary filter. Otherwise, a combination of a primary filter made of Al (500 μm thickness) and an X-ray tube voltage of 40 kV was the better option for the determination of other elements with higher energy emission lines (Mn, Fe, Cu, Zn, Rb and Sr). Considering that the aim of the study was multielemental analysis of coffee samples finally it was decided to select the use of a primary filter made of Al (500 μm thickness) and an X-ray tube voltage of 40 kV to perform coffee sample analysis.

3.3. Quantification EDXRF approaches

Due to the absence of commercially available coffee reference materials, the use of a standard-less procedure based on fundamental parameters (FP) was evaluated as a possible quantification mode. The FP approach provided in the S2 EDXRF Ranger software was selected for this purpose. This method computes the element concentrations through a fitting model which compares the experimental spectra with a theoretical one. Some parameters regarding sample preparation (sample diameter, amount of mass and thickness) can be included in the model to improve the fitting. It is also possible to include the molecular formula of the sample matrix to better compensate for sample self-absorption and enhancement effects. In this study, the composition of the sample matrix was estimated from the ratio of measured scatter peak intensities. An experimental calibration of the ratio of incoherent to coherent Pd (X-ray tube anode) scatter peaks for measured compounds having an effective atomic number (Z_{eff}) between 6.16 and 14.65 served to establish an equation to determine the effective atomic number for unknown samples (see Fig. 2). Z_{eff} was calculated using the following expression:

$$Z_{\text{eff}} = \frac{\sum (W_k Z_k A_k)}{\sum (W_k A_k)}$$

where: W_k is the percentage of mass of the element k in the compound, Z_k is the atomic number of the element k and A_k is the atomic mass of the element k .

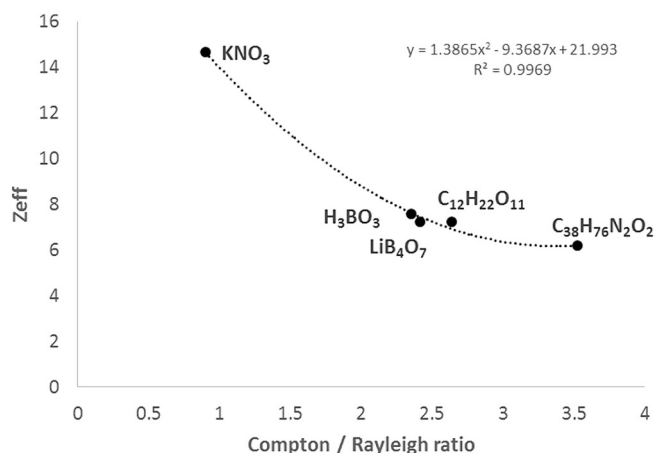


Fig. 2. Experimental calibration of scatter ratio. Z_{eff} of compounds was calculated according the expression: $Z_{\text{eff}} = \frac{\sum (W_k Z_k A_k)}{\sum (W_k A_k)}$, where W_k is the percentage of mass of the element k in the compound, Z_k is the atomic number of the element k and A_k is the atomic mass of the element k .

Later, Z_{eff} was estimated for each type of coffee sample under consideration. No statistically significant differences were obtained among the Z_{eff} values for different coffee types: Pure coffee ($Z_{\text{eff}} = 6.92 \pm 0.16$, $n = 10$), instant coffee ($Z_{\text{eff}} = 7.01 \pm 0.13$, $n = 2$) and coffee with additives ($Z_{\text{eff}} = 6.88 \pm 0.19$, $n = 7$). Therefore, a mean Z_{eff} value was estimated ($Z_{\text{eff}} = 6.916$, $n = 19$) and it was used to define a matrix composed of a combination of elements producing such an effective atomic number ($\text{C}_{25}\text{H}_{32}\text{N}_{10}\text{O}_{12}$). This hypothetical composition was later included in the quantification model as the coffees' matrix.

To study the quality of the results obtained using the FP method, a set of eleven coffee samples was also analyzed by digestion and ICP-OES analysis (details in section 2.3.2) and results were compared with those obtained by FP-EDXRF using a linear regression method. For the comparison R^2 , intercept and slope were evaluated. If results for both methods are equal these parameters should not be significantly different from $R^2 = 1$, slope = 1 and intercept = 0. In Fig. 3 regression plots

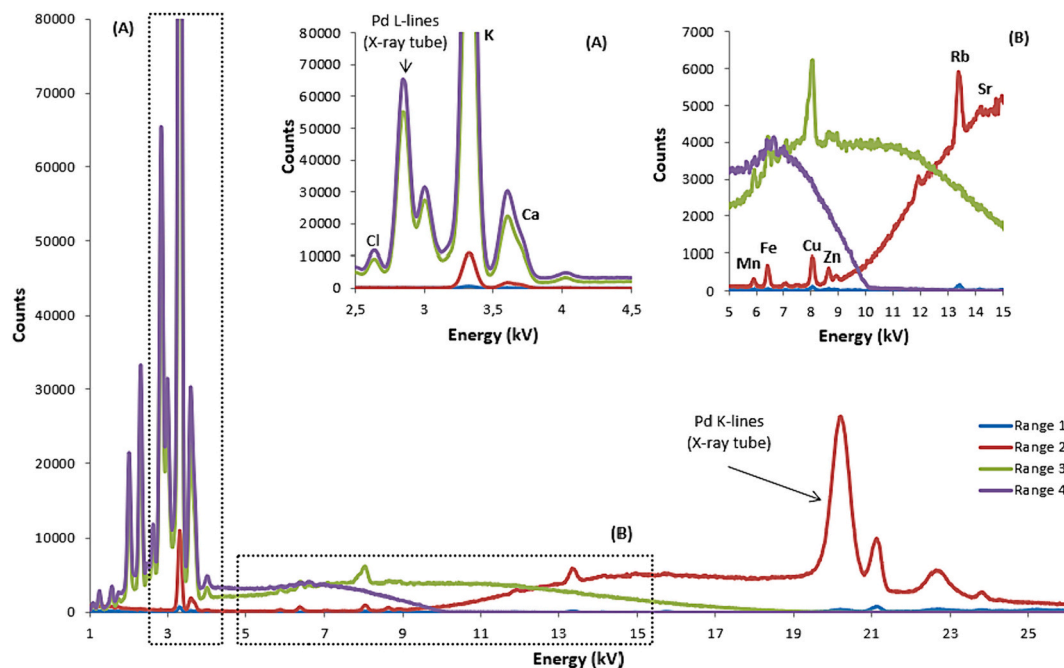


Fig. 1. EDXRF spectra showing the influence of measurement conditions on multielemental analysis of coffee samples (Range 1: 50 kV Cu filter 100 μm , Range 2: 40 kV Al filter 500 μm , Range 3: 20 kV without filter, Range 4: 10 kV without filter).

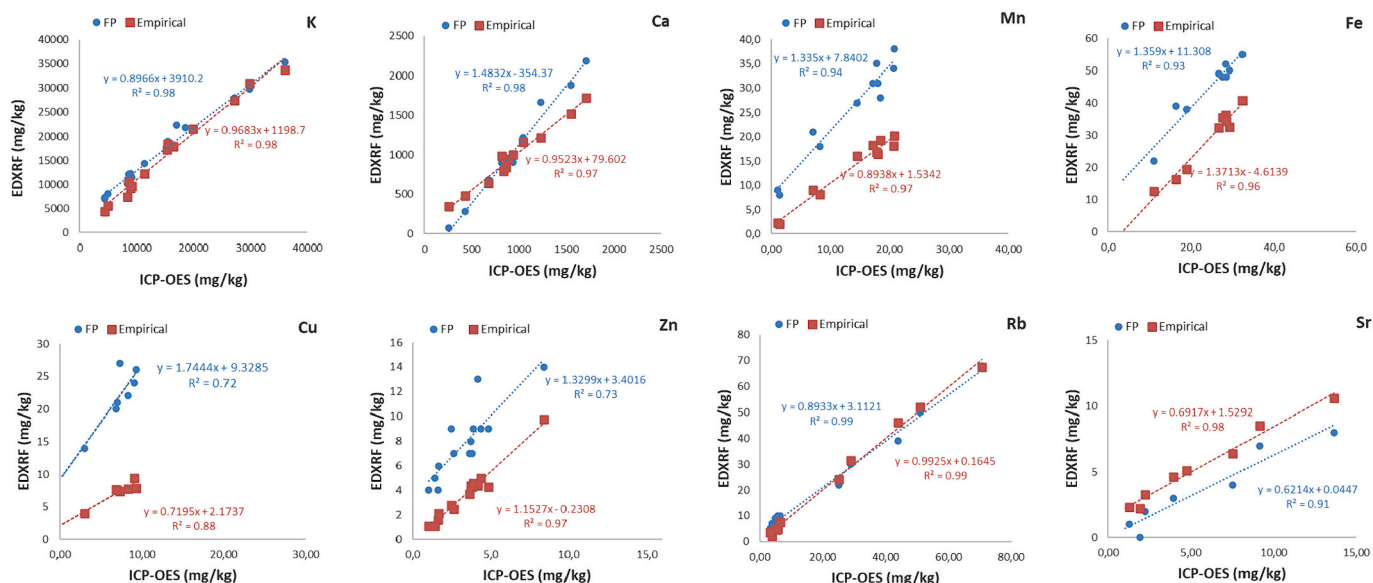


Fig. 3. Regression plots correlating results for coffee analysis by ICP-OES versus EDXRF using different quantification approaches (FP: Fundamental parameters, Empirical: Empirical calibration).

correlating results for coffee analysis by digestion + ICP-OES and FP-EDXRF are displayed. In general, an acceptable linearity was obtained for most elements (except for trace elements such as Cu and Zn) but slope and intercept values were significantly different from 1 and 0, respectively.

In view of the obtained results by FP-EDXRF method another quantification approach, based on the use of empirical calibration, was also explored. Empirical calibrations were established using a set of 11 coffee samples covering the whole range of element concentrations (see Table 1 for details). For all analytes, calibrations were based on a regression plot of corrected analyte intensity (compensating for the inter-elemental effects and line overlapping) versus concentration (reference value obtained by digestion + ICP-OES analysis). A good linearity was obtained for most of the studied elements (see R² and standard error of calibration (SEC) in Table 1). Limits of quantification for all elements were < 5 mg kg⁻¹, except for K and Ca which were higher (50–180 mg kg⁻¹).

A validation step was then performed using the obtained calibration models to determine analyte concentrations in a second set of samples totally independent of the calibration set. These samples were the same ones previously quantified using the FP-EDXRF method. Results are displayed in Fig. 3. As it is shown, a clear improvement of results was assessed when using the empirical calibration method, above all for some elements such as Cu, Zn and Fe. Surely the reason for that is due to the identifiable small peaks of these elements arising from the

background signal received from the equipment design (instrumental blank).

Therefore, empirical calibration was finally selected as the quantitative mode for coffee analysis by EDXRF.

3.4. Chemometric strategies in combination with EDXRF data for coffee type classification

After the development of the EDXRF method for coffee analysis, different exploratory and discriminant chemometric techniques (principal component analysis, PCA and partial least squares discriminant analysis, PLS-DA) were applied to the obtained data to assess their potential for classifying different types of coffee (pure coffee, instant coffee and coffee with additives).

PCA is one of the most commonly used chemometric tools in combination with EDXRF to discriminate sample types (according to their origin, quality, species, variety, etc) in food science and nutrition [25,26]. In the present study, element concentrations (K, Ca, Mn, Fe, Cu, Zn, Rb and Sr) determined using the empirical EDXRF method were used as variables. Prior to statistical analysis, element contents were standardized to compensate for the different scales. As it is shown in Fig. 4A, three principal components (PCs) were extracted from the dataset, which explained 86.1% of the total variability. According to the loadings of the variables for the PCs, the most significant PC1 was strongly associated with all the variables (especially Mn concentration) but Ca and Zn concentrations. The concentrations of Ca and Zn constitute the information of the second principal component (nearly 50% for each element). The principal contributions of the third principal component come mainly from Rb (with a negative correlation) and then from K (negative correlation), Fe and Cu.

The score plot (Fig. 4A) showed a good clustering of coffee samples depending on their type (pure coffee, instant coffee and coffee with additives). In the case of coffee samples with additives group, a higher dispersion of samples can be observed in comparison with pure and instant coffee ones. This fact can be related to the inherent elemental composition variability of the coffee additives (milk, chocolate, vanilla, Irish cream and hazelnut).

As an alternative, to speed up the whole analytical process, the application of PCA directly on the obtained raw EDXRF spectrum (without the need for fitting and quantification) was also tested. In this case, 1570 channels (ranging from 0.62 up to 32 keV) extracted from the

Table 1

Concentration range of elements in calibration coffee samples and values for linear regression analysis between EDXRF data and reference values (digestion + ICP-OES).

Element	Min (mg kg ⁻¹)	Max (mg kg ⁻¹)	n	R ²	SEC	LOQ (mg kg ⁻¹)
K ¹	0.34	3.58	11	0.99	0.0978	0.018
Ca ¹	0.0246	0.1854	11	0.97	0.0075	0.005
Mn	0.84	16.62	10	0.95	1.41	2.0
Fe	3.3	33.7	8	0.99	3.12	2.4
Cu	0.34	9.89	7	0.99	1.20	1.1
Zn	0.945	13.6	9	0.98	0.55	0.7
Rb	3.22	98.21	9	0.99	3.63	2.7
Sr	1.25	22.57	10	0.90	2.80	4.5

¹ Concentration values in percentage (g 100 g⁻¹), SEC: Standard error of calibration [27].

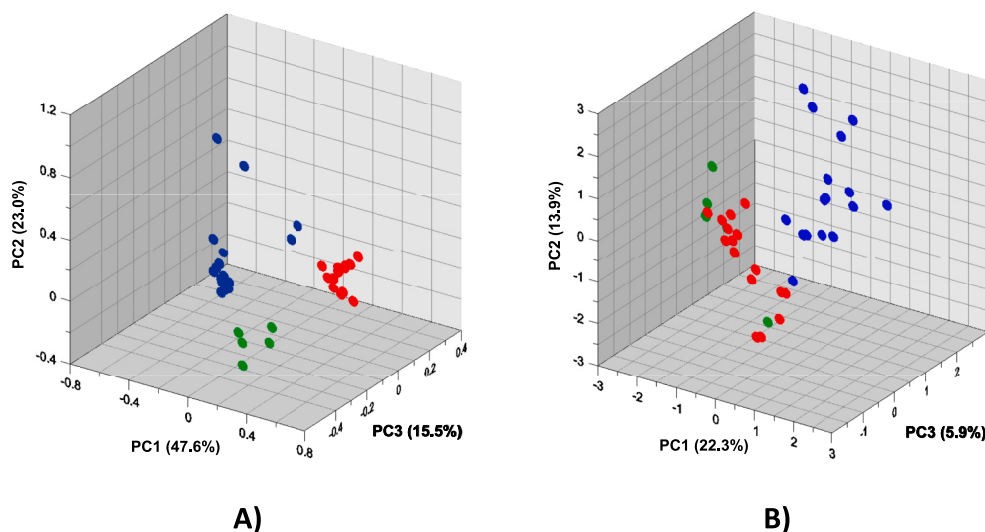


Fig. 4. Principal component analysis (PCA) score plot derived from: (A) element concentrations (K, Ca, Mn, Fe, Cu, Zn, Rb and Sr) determined in coffee samples by empirical calibration and (B) EDXRF analysis from the whole EDXRF spectrum information (blue color stands for coffee with additives, green are for instant coffee and red means pure coffee). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

EDXRF spectrum were selected as variables. In Fig. 4B, the obtained scores plot is displayed. As it is shown, in this case, the three PCs explained only 42.1% of the total variability and the clustering between sample groups is not as good as the one obtained when using element concentration as variables. To improve sample classification using EDXRF spectra, a partial least squares discriminant analysis (PLS-DA) was developed and validated. In all the calculations, four LVs were extracted. The training (fitting) calculation gave a very good result where all the samples were correctly classified in their group. To evaluate the reliability of the model the obtained sample classification was tested by performing predictions. This has been conducted by means of a leave-one-out (LOO) calculation. This procedure consists of predicting the membership of each sample by means of excluding it from the data, building a model with the remaining 36 samples and, finally, applying the model to the removed item. The procedure demands to build a total of 37 PLS-DA models, one per predicted sample.

In Fig. 5 the PLS-DA-LOO results are shown. As mentioned above, each sample is depicted in Fig. 5 according to a model composed by two discriminant functions which are distinct for all the samples (because each classification model is obtained from a different training calculation involving a slightly distinct fitting sample set). For each predicted sample a first classification function (Function A) is applied. If the value obtained is >0.5 the sample is classified as a type A coffee (i.e., with additives). If the value is less than 0.5, then another discriminant function (Function I) is considered. Again, if the value obtained for this function is >0.5 the sample is classified as a type I coffee (instant). In the other cases the sample is classified as P type (pure). The figure shows a good clustering (function limit values have been depicted as frontier thresholds), where the three coffee types are separated. In this case, no errors of classification are found. This fact confirms that the coffee sample types considered can be directly classified according to their EDXRF spectra in combination with PLS-DA without the need to use element concentrations. Despite the fact that additional research is needed to test this approach in other types of food varieties, this opens up the potential use of EDXRF spectrum and PLS-DA as an attractive tool in food traceability.

4. Conclusions

In this work, an EDXRF method has been developed for elemental composition determination (K, Ca, Mn, Fe, Cu, Zn, Sr and Rb) in different type of coffee samples. The method is easy and fast and consists

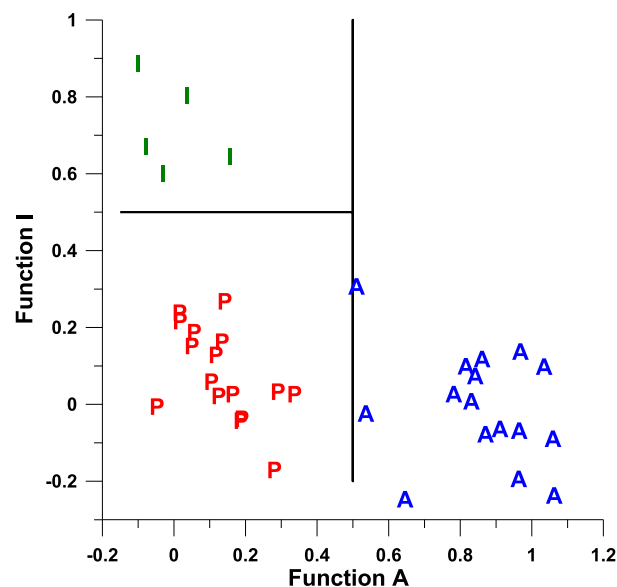


Fig. 5. Classification of the samples by means of a leave-one-out procedure (I: Instant coffee, P: Pure coffee, A: Coffee with additives) using PLS-DA from the full raw spectrographic data. Each sample group prediction has been obtained from a different pair of classification functions A and I (see the text for more details).

of pelletizing 2 g of the powder material with 0.2 g of wax and using an empirical calibration of the spectrometer with a set of characterized coffee samples (previously analyzed by digestion and ICP-OES).

Results from the different chemometric approaches explored for coffee type classification (pure, instant and with additives) demonstrated that the application of PLS-DA on the raw EDXRF spectra can be a promising approach. In this case, fitting the EDXRF spectra and the quantifying the elemental concentrations, which are typically used as variables in PCA analysis, are not necessary.

All in all, it is expected that the results derived from this work can be useful to establish a reliable and simple method for multielemental coffee analysis using EDXRF. In addition, our approach provides a good basis for the further application of EDXRF spectra as sample fingerprints in other food traceability and/or authentication studies in the future.

CRedit authorship contribution statement

M. Weinberger: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition. **I. Queralt:** Writing – review & editing, Methodology. **C. Strel:** Writing – review & editing, Supervision. **P. Wobrauschek:** Writing – review & editing, Supervision. **E. Besalú:** Writing – review & editing, Writing – original draft, Software, Methodology. **J. Jablan:** Writing – review & editing, Investigation, Conceptualization. **E. Marguí:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Supervision, Conceptualization.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

Acknowledgments

Matthias Weinberger acknowledges the Erasmus+ Program (Student mobility for studies/traineeships-2022/23). Publication fees funding was provided by CRUE-CSIC agreement with Elsevier in the acknowledgments sections.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.sab.2024.106898>.

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