Physico-elemental analysis of roasted organic coffee beans from Ethiopia, Colombia, Honduras, and Mexico using X-ray micro-computed tomography and external beam particle induced X-ray emission

Karen J. Cloete¹, Žiga Šmit²,³, Roya Minnis-Ndimba¹, Primož Vavpetič², Anton du Plessis⁴, Stephan G. le Roux⁴, Primož Pelicon²

¹iTemsba Laboratory for Accelerator Based Sciences, National Research Foundation, PO Box 722, Somerset West 7129, South Africa
²Jožef Stefan Institute, Jamova cesta 39, SI-1001 Ljubljana, Slovenia
³Faculty of Mathematics and Physics, University of Ljubljana, Jadranska ulica, 19, SI-1000 Ljubljana, Slovenia
⁴CT Scanner Facility, Central Analytical Facilities, Stellenbosch University, Private Bag X1, Matieland, Stellenbosch 7602, South Africa

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ABSTRACT

The physico-elemental profiles of commercially attained and roasted organic coffee beans from Ethiopia, Colombia, Honduras, and Mexico were compared using light microscopy, X-ray micro-computed tomography, and external beam particle induced X-ray emission. External beam PIXE analysis detected P, S, Cl, K, Ca, Ti, Mn, Fe, Cu, Zn, Br, Rb, and Sr in samples. Linear discriminant analysis showed that there was no strong association between elemental data and production region, whilst a heatmap combined with hierarchical clustering showed that soil-plant physico-chemical properties may influence regional elemental signatures. Physical trait data showed that Mexican coffee beans weighed significantly more than beans from other regions, whilst Honduras beans had the highest width. X-ray micro-computed tomography qualitative data showed heterogeneous microstructural features within and between beans representing different regions. In conclusion, such multidimensional analysis may present a promising tool in assessing the nutritional content and qualitative characteristics of food products such as coffee.

1. Introduction

Coffee is a one of the most popular and desired beverages consumed by millions of people worldwide for its hedonistic and psychostimulant benefits (Labbe, Ferrage, Rytz, Pace, & Martin, 2015). Coffee is also used in the pharmaceutical, cosmetic, and food industries (Mazzafera, 2012). Based on the popularity of coffee in these industries, assessing the nutritional content, quality, and geographic origin of beans is important (Liu, You, Chen, Liu, & Chung, 2014). In assessing coffee bean nutritional content, quality, and geographic origin, exploring the use of a multidimensional approach involving bean physico-elemental analysis may hold value.

The elemental profile of coffee beans has been routinely assessed using a range of traditional analytical techniques to assess its nutritional content and geographic origin (Zaidi, Fatima, Arif, & Qureshi, 2005; Filho, Polito, & Neto, 2007; Grembecka, Malinowska, & Szefer, 2007; Liu et al., 2014). Most of these elemental screening techniques however employ bulk chemical processing that requires tedious preparation with harmful chemicals that may introduce contaminants and processing artefacts. Alternatively, achieving a homogeneous particle size and distribution for non-chemically processed ground samples significantly depends on the structural characteristics of the specimen and the milling material used for comminution (Santos et al., 2008). Most importantly, the sample matrix is dissolved or pelletized, destroying important information on quantitative elemental distribution and sample microstructure (Oas Graças Andrade Korn et al., 2008).

Proton induced X-ray emission (PIXE) has been described as one of the most promising and versatile elemental screening techniques (Nastasi, Mayer, & Wang, 2014) based on its ability to quantify elements heavier than Na at µg/g-level sensitivity in samples that require minimal processing (Johansson, Campbell, & Malmqvist, 1995). Furthermore, as elemental distribution mapping in intact samples is

Abbreviations: PIXE, particle induced X-ray emission; LDA, linear discriminant analysis; micro-CT, X-ray micro-computed tomography

⁎ Corresponding author.

E-mail addresses: kcloete@tlabs.ac.za (K.J. Cloete), ziga.smit@fmf.uni-lj.si (Ž. Šmit), rminnis@tlabs.ac.za (R. Minnis-Ndimba), primoz.vavpetic@ijs.si (P. Vavpetič), anton2@sun.ac.za (A. du Plessis), lerouxsg@sun.ac.za (S.G. le Roux), primoz.pelicon@ijs.si (P. Pelicon).

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possible, PIXE may find novel applications in food chemistry research. The application of PIXE in food chemistry research has however been rather limited, with only a few publications focused on the elemental analysis of conventionally produced coffee (Debastiani, dos Santos, Yoneama, Amaral, & Dias, 2014; Debastiani et al., 2019). Performing PIXE with an external beam may hold even more promise since quantitative elemental mapping of larger intact and hydrated samples under non-vacuum conditions is possible that allows negligible charging of the insulating targets, reduced radiation damage and loss of volatile elements, and simple handling and changing of samples (Giuntini, 2011).

Besides elemental analysis, physical characterization of coffee beans may also define its qualitative characteristics. Traditionally, such characterization has been carried out in the past using light microscopy, electron microscopy, and mercury porosimetry (Schenker, Handschin, Frey, Perren, & Escher, 2000). Similar to traditional elemental screening techniques, these methods require time-consuming sample preparation with hazardous chemicals that may introduce processing artefacts, whilst bulk physical and internal microstructural screening of samples is also not possible. There is hence a need to explore techniques that allow for the bulk assessment and internal 3-dimensional characterization of coffee beans to establish a fundamental correlation between microstructure and quality. For this purpose, X-ray micro-computed tomography (micro-CT or μCT) presents an attractive and innovative radiographic imaging tool to aid the non-destructive and non-invasive 2 and 3-dimensional microstructural analysis of food products at atmospheric temperature and pressure (Schoeman, Williams, du Plessis, & Manley, 2016). To date, no publications exist on the application of micro-CT to study the qualitative characteristics of roasted coffee beans from different regions based on its microstructural features.

There are currently no reports providing bulk multidimensional elemental, physical, and microstructural data derived from intact and roasted organic coffee beans to assess its nutritional content and qualitative characteristics at the consumer level. The aim of this study was hence to screen commercially available and intact roasted organic coffee beans from some of the world’s top coffee production regions to assess bean nutritional content and qualitative characteristics utilizing physico-elemental data that may also geographically authenticate beans. Specific objectives of this study were to 1) quantitatively screen and compare the elemental content of roasted organic coffee beans from Ethiopia, Colombia, Honduras, and Mexico using external beam PIXE and 2) quantitatively screen and compare the physical properties of beans using light microscopy; and 3) qualitatively screen and compare the microstructural properties of beans using X-ray micro-computed tomography.

2. Experimental

2.1. Sample collection and preparation for external beam PIXE analysis

Coffee samples were sourced locally and included commercially available roasted organic coffee beans from Ethiopia, Colombia, Honduras, and Mexico. The beans were not chemically processed and only cross-sectioned using a stainless steel razor blade to expose its morphological regions. Sectioned beans were introduced into the external beam PIXE setup of the Jožef Stefan Institute, Slovenia (13 replicates per sample representing one region).

2.2. External beam PIXE analysis

A mechanical holder containing a single cross-sectioned bean was mounted at a fixed distance from the proton exit window and the detector. The same area on all samples was subsequently measured with a proton beam of initial energy 3 MeV that decreased to approximately 2.77 MeV on the target due to energy loss in the air and exit window. All samples were measured with an ion current of 1–3 nA.

The energy range of the detected X-rays was set between 1.5 and 30 keV. Emitted X-rays were detected by a Si(Li) detector with a resolution of 160 eV at 5.89 keV and positioned 6.1 cm from the target at a 135° angle according to the beam direction. Apart from the air space between the sample and detector, the X-rays passed a pinhole (funny) filter of 50 μm aluminum foil for which the fitted transmission function yielded an effective thickness of 45 μm and a relative opening of 0.0856. The funny filter provided a good balance between the intense peaks of potassium and low intensity peaks of metal elements.

The spectra were normalized according to the argon signal induced in the air space between the window and target. A part of the argon signal was screened by mechanical obstacles, which we determined semi-empirically. The spectral fitting was performed by the AXIL code and elemental concentrations determined by the model of independent physical parameters calibrating the geometrical parameters from a set of measurements on pure elemental or simple chemical compound targets. For test measurements, we measured the glass standard NIST 620 as an unknown target, whilst for the analysis of coffee grains the analysed elements were assumed to be imbedded in a cellulose matrix.

2.3. Quantitative measurements of physical traits of interest

Bean mass, length, width, and thickness were determined for 100 samples from each region using the method described in Ismail, Anuar, and Shamsudin (2013). Bean mass was determined using a digital balance with an accuracy of 0.0001 g. This process was repeated three times and the mean value for the data from the three repetitions used as the final mass value for a specific region (Bart-Plange & Baryeh, 2003). Light micrographs of beans were captured using a DS-F12 digital camera mounted on a Nikon SMZ 1500 trinocular stereomicroscope. Associated NIS software tools were used to measure the main axial dimensions of bean length, width, and thickness. The average volume of the beans was calculated from measurements of the major, minor, and intermediate diameters, following the assumption that each bean can be described as half a trachial ellipsoid (Dutra, Oliveira, Franca, Ferraz, & Afonso, 2001) using Eq. (1)

\[ V = \frac{2}{3} \pi abc \]  

(1)

where a, b, c are the half-length, half-width, and thickness of the bean, respectively.

2.4. micro-CT analysis

Individual beans were attached to plastic rods affixed to dense polystyrene foam platforms to ensure sample rigidity. The beans were consequently scanned at 20 μm and 35 μm isotropic voxel resolution using the General Electric Phoenix v’TomeX L240 with NF180 option and the General Electric Phoenix Nanotom S systems (du Plessis, le Roux, & Guelpa, 2016). Previously described methods were used for scanning parameter optimization and included X-ray spot size settings within a selected scan resolution and high transmitted brightness values in the live digital X-ray images that indicated good X-ray penetration (du Plessis, Broeckhoven, Guelpa, & le Roux, 2017). The X-ray voltage was set between 60 and 100 kV and current from 100 to 200 μA, whilst a 0.1 mm copper beam filter was used in the 100 kV scans to reduce potential beam hardening artifacts. High quality data acquisition and good image contrast were ensured by background detector calibrations and activated detector shifts to minimize ring artefacts. In each 360° sample rotation, between 1600 and 3100 images were captured in steps with an image acquisition rate of 500–1000 ms/image and no averaging of images. The acquired projection images were reconstructed using General Electric Datos software and analyzed using Volume Graphics VG Studio Max 3.0 (Heidelberg, Germany). Images were segmented to remove exterior air and the defect analysis function was applied to...
show the microporosity distribution in 2D and 3D images. We subsequently used qualitative analysis of 2D and 3D images to assess microstructural differences related to the distribution and features of pores in beans representing different geographical regions. Major cracks and the void where the tegumentum resides were excluded from the computational analysis.

### 2.5. Statistical analyses

Descriptive statistics (mean ± SD) was used to compare elemental data, whilst Tukey’s HSD was used to compare physical trait data. Elemental data of samples collected from the different geographical areas was graphically depicted using exploratory analysis with a heatmap combined with hierarchical clustering and linear discriminant analysis (LDA). Only elements detected in all samples were included in the heatmap combined with hierarchical clustering and LDA analyses.

A heatmap was constructed for the median profiles in clusters using the heatmap function in the R 3.4.2 software and served as both a visualization and partitioning tool. Dendrograms were added to the margins of the heatmap using hierarchical clustering with a complete linkage method based on the Euclidean distances among clusters in rows or among elements in columns. The experimental data was standardized and scaled prior to heatmap analysis as wide differences in data dimensionality may lead to misclassification (Liu, Lin, & Kuo, 2003).

To find a linear combination of the explanatory variables that best differentiates between the groups, LDA was used. The model is based on the calculation of the total and within sample matrix. In our developed code, we used Cholesky decomposition, thus reducing the eigen-value problem to the symmetric case. The results display the data for concentration to the symmetric case. The results display the data for the first two canonical variables, which retain 99.04% of the initial variance:

\[
Z_1 = 8.7303 c - 5 * [P] - 2.8718 e - 4 * [S] + 8.6312 c - 4
\]

\[
* [Cl] + 7.4957 c - 6 * [K] + 7.7181 c - 5 * [Ca]
\]

\[
Z_2 = -4.2517 e - 5 * [P] + 2.4698 e - 4 * [S] + 2.2448 e - 4
\]

\[
* [Cl] - 1.2884 c - 5 * [K] - 1.2153 c - 4 * [Ca]
\]

where the square brackets denote the elemental concentrations in µg/g.

A similar diagram was calculated for minor elements with the first two canonical variables that keep 85.15% of the variance:

\[
Z_1 = 6.7242 c - 4 * [Mn] - 3.1977 e - 4 * [Fe] - 1.0302 c - 3
\]

\[
* [Cu] + 2.0943 e - 2 * [Zn] + 7.1130 c - 3[Rb]
\]

\[
Z_2 = 1.1151 e - 2 * [Mn] - 1.0704 e - 2 * [Fe] + 1.0834 c - 2
\]

\[
* [Cu] + 3.4293 c - 2 * [Zn] - 2.936 e - 3[Rb]
\]

### 3. Results

Relatively high levels of the macro-elements P, S, Cl, K, Ca, and relatively low levels of the micro-elements Mn, Fe, Cu, Zn, Rb, and Sr were detected in all beans (Table 1). Some beans also contained trace levels of Br and Ti. Elemental maps with the highest statistics showed an inhomogeneous distribution pattern for K and Fe, with the highest concentrations of K and Fe located within the endosperm region (Fig. 1) that was selected for elemental profiling of all beans.

Elemental levels detected in all beans are reported in Table 1 (Cloete, Šmit, Minnis-Ndimba, Vavpetič, & Pelicon, 2018). There was no difference between the elemental content of beans from different regions based on descriptive statistics. Beans from Mexico, Colombia, Honduras, and Colombia contained the highest concentrations of Cl, Mn, Fe, and Sr, respectively, whilst beans from Colombia, Ethiopia, Mexico, and Ethiopia contained the lowest concentrations of S, Mn, Rb, and Sr, respectively.

| Macro-elements | Columbia | Honduras | Mexico | Ethiopia |
|----------------|----------|----------|--------|----------|
| P              | 766 ± 521| 977 ± 665| 900 ± 319| 995 ± 643|
| S              | 1328 ± 397| 1934 ± 694| 1724 ± 401| 1936 ± 594|
| Cl             | 309 ± 175| 390 ± 270| 662 ± 184| 527 ± 111|
| K              | 16543 ± 3632| 16805 ± 4503| 17577 ± 2265| 17315 ± 2610|
| Ca             | 1686 ± 758| 1371 ± 391| 1574 ± 960| 1205 ± 447|

Hierarchical clustering showed the similarity or differences in elemental composition of beans based on origin and the similarity or differences between regions based on bean elemental content. The color legend of the heatmap (Fig. 2a) indicates a negative/lower average elemental concentration (blue) or a positive/higher average elemental concentration (red) in a particular sample.

Honduras showed above average z-scores and Mexico below average z-scores. The highest dissimilarity to average values (extreme z values as shown by the brightest colours of the colour map) was observed for the z-scores of Colombia and Mexico. Cluster analysis of the regions provided a dendrogram clustering Honduras and Ethiopia, with Colombia separated from all other regions. The cluster analysis of the elements showed two large clusters. The first large cluster grouped Cl, K, S, and P, whilst the second large cluster was divided into a group containing Rb and Fe, and two additional groups containing Ca and Cu, and Mn and Zn, respectively. LDA showed that there was no significant association between elemental levels and geographical origin (Fig. 2b).

Beans from Ethiopia weighed significantly (p = 0.00) less than beans from other regions, whilst Mexican beans weighed significantly (p = 0.00) more than beans from other regions (Mean width [mm] ± SD, n = 100: Ethiopia: 0.11 ± 0.003; Colombia: 0.13 ± 0.003; Honduras: 0.13 ± 0.001; Mexico: 0.15 ± 0.004). Bean width significantly (p = 0.00) differed between all four regions, with Honduran beans having the highest width (Mean width [mm] ± SD, n = 100: Colombia: 7.62 ± 0.59; Ethiopia: 7.19 ± 0.49; Honduras: 8.16 ± 0.53; Mexico: 7.78 ± 0.59).

X-ray micro-computed tomography images were of high quality showing no analytical artefacts (Fig. 3). Fig. 4 depicts highly contrasted 2D images of the reconstructed sagittal and transverse plane of beans from different regions (Mexico, Colombia, and Honduras). The 2D and 3D images (not shown) revealed a spatial distribution map of differences in X-ray absorption across bean morphological regions of varying structure. Brighter regions with higher X-ray attenuation coefficients correspond to high density areas or the vitreous matrix of the bean, whilst darker regions with lower X-ray attenuation coefficients correspond to lower density areas or bean macro and micro-cavities (Fig. 4, gray-scale images).

For qualitative analysis of bean porosity, 2D images were selected since the microporosity was too extensive and not possible to visualize clearly for the entire bean. The 2D images (Fig. 4) showed a uniform distribution of pores but low homogeneity in pore features and distribution in morphological regions of beans representing different regions. More specifically and upon closer inspection, “larger pores” revealed pore interconnectivity that was highest around void regions in all beans (Fig. 4, regions marked in red). Ethiopian beans showed the highest pore interconnectivity (Fig. 5, regions marked in red). These interconnected pores seem to be the result of fused individual pores.
4. Discussion

This is to our knowledge the first report describing a multi-dimensional analytical approach exploiting physico-elemental analysis to screen the nutritional content and qualitative characteristics of commercially available roasted organic and non-chemically processed coffee beans from some of the top coffee production regions.

Elemental data showed that beans from all regions contained the macro-elements P, S, Cl, K, and Ca and the micro-elements Zn, Cu, Fe, and Mn. The macro-element profile for all beans showed mean concentrations in the order of: K > S > Ca > P > Cl and a micro-element profile in the order of: Rb > Fe > Mn > Cu > Sr > Zn. Colombian beans may present with a higher nutritional value due to its higher levels of Zn, Ca, Mn, Rb, and Sr, whilst Ethiopian beans may present with a higher organoleptic quality characteristic due to its elevated S levels.

The same range of elements reflecting a nutritional and organoleptic qualitative profile important for human health and vitality (Şemen, Mercan, Yayla, & Açikkol, 2017) was detected by traditional elemental analytical methods in conventionally farmed coffee beans from the same geographical regions (Liu et al., 2014; Habte et al., 2016). No toxic metals were detected in the samples, although Habte et al. (2016) reported levels well below the short-term tolerable intake values for arsenic, mercury, cadmium, and other toxic metals in conventionally farmed Ethiopian green coffee beans. The presence of trace levels of Ti and Br in organically farmed Ethiopian and Mexican coffee beans used in this study was surprising and may be ascribed to environmental pollution or the unscrupulous use of Br or organotin-based pesticides (Pérez-Olivera, Navarro-Garza, & Miranda-Cruz, 2011). Nevertheless, previous studies have similarly reported negligible amounts of toxic metals in organic foods (Mie et al., 2017).

Statistical analysis of bean elemental data with a heatmap showed that Colombian beans were differentiated from beans from Mexico, Honduras, and Ethiopia by more extreme z-scores in the negative direction for macro-elements (except for Ca) and more extreme z-scores in the positive direction for micro-elements (except for Rb). Honduras showed a strong positive signal for Ca and Mexico a strong negative signal for Rb. Interestingly, the clustering of elements did not resemble a profile characteristic of bean physiological needs, but rather suggests that soil-plant physico-chemical properties may play an important role in determining bean elemental profile. To illustrate, since Ca was separated from other macro-nutrients, Ca availability in soils of the representative regions may be higher. Regional soil-plant physico-chemical properties may also explain the clustering of Ethiopia with Central (Honduras) and North-American (Mexico) countries. The contents of macro and micro-elements may hence not be significant for particular regions as also shown by the weak association between geographic origin and bean elemental content in LDA analysis. Nevertheless, LDA showed some slight regional differences for micronutrient content, with Colombia partly separated from other regional groups and Mexico more tightly grouped than other regions. This is not surprising as coffee bean elemental content may not only be influenced by geographical location and soil physico-chemical properties, but also by bean variety, production site environmental factors including climate (Anderson & Smith, 2002), agricultural practices including organic versus conventional farming (Velmourougane, 2016), processing conditions during roasting (Cruz, Morais, & Casal, 2015), and sample processing and analytical methodology employed (Das Graças Andrade Korn et al., 2008; Cruz et al., 2015).

Comparison of elemental data using descriptive statistics however showed that beans from the selected regions could be partly differentiated by their elemental content. For example, Mexican beans contained the highest levels of Cl, an element that imparts a musty and moldy odor to coffee (Kato, Sato, & Takui, 2011). Moreover, Ethiopian beans had the highest levels of S, another key element defining the organoleptic profile of coffee (Ojha & Roy, 2018). Interestingly, Colombian beans had the highest levels of Rb and Sr, whilst Ethiopian beans had the lowest levels of Sr. Interestingly, the presence of Rb and Sr in beans may be related to regional soil physico-chemical characteristics (Liu et al., 2014) as also illustrated by other studies who reported that green and roasted Brazilian beans contain relatively high concentrations of Rb, reflecting its high levels in Brazilian soils (Debastiani et al., 2014). Consequently, elements such as Rb and Sr play a significant role in the geographic authentication of plant food

Fig. 1. Elemental distribution maps of K, Ca, and Fe of a hand-sectioned roasted organic coffee bean (top image). The intensity scale in maps was selected to emphasize certain elemental distribution features.
products (Debastiani et al., 2014; Liu et al., 2014).

Alternatively, Rb and Sr may also play an important role in human health, with Rb overexposure resulting in mineral imbalances of K associated with neuromuscular symptoms, hyperirritability, weight loss, and skin ulcers, whilst Rb deficiency is associated with the development of atherosclerosis (Moustafa, 2015). Interestingly, Sr has been shown to play an important role in bone development (Sila-Asna, Bunyaratvej, Maeda, Kitaguchi, & Bunyaratavej, 2007).

Besides the role of bean elemental profile in determining nutritional qualitative and geographic characteristics, elemental levels in beans may also define the physical properties classifying coffee’s economic value, with smaller beans normally having a lower market value (Barel & Jacquet, 1994; Kitila, Alamerew, Kufa, & Garedew, 2011). Physical trait analysis showed that Mexican beans weighed significantly more compared to beans from other regions, whilst Honduras beans had the highest width. Interestingly, bean size may be linked to the altitude or shading of the coffee plantation, with increased bean sizes normally observed for plants grown at high altitude or in the shade (Tolessa, D'heer, Duchateau, & Boeckx, 2017). However, increases in bean size and weight may also be observed in beans with higher moisture levels influenced by weather or storage conditions (Tharappan & Ahmad, 2006).

Besides the elemental and bulk physical characteristics of coffee, its microstructural characteristics may also define its qualitative profile. To illustrate, coffee beans undergo significant changes during roasting when pores and cracks of differing geometry develop across the bean due to the effects of heat and mass transfer produced by pyrolytic reactions and thermal degradation of organic components (Oliveros, Hernández, Sierra-Espinosa, Guardián-Tapia, & Pliego-Solórzano, 2017). These microstructural changes significantly affect the chemistry, organoleptic properties, and stability of the consumer product by controlling mass transfer phenomena, gas absorption and degassing capacity, the release of mobilized oil from the bean surface, as well as the susceptibility of the bean to fungal infections and imbibition damage (Schenker et al., 2000). Undesirable changes in bean microstructural properties induced by roasting may hence be directly responsible for changes in the qualitative characteristics of coffee in terms of its organoleptic profile and shelf life (Schenker et al., 2000). Interestingly, changes in the microstructural porous structure of coffee also affects its mechanical properties (such as brittleness) that determine the
efficiency of grinding and the extraction yield of soluble solids during brewing (Pittia, Dalla Rosa, & Lerici, 2001).

Our data showed that beans from different regions were characterized by distinct microstructural differences in terms of pore features and distribution. Such variation could be attributed to the thermal intensity of the roasting treatment applied or varying moisture levels in beans from different regions (Schenker et al., 2000). Ethiopian beans exhibited higher pore interconnectivity, with individual and interconnected pores inhomogeneously distributed across bean morphological regions. Ethiopian beans may hence present with a shorter shelf-life, but may be easier to grind.

5. Conclusions

External beam PIXE provided both quantitative data on elemental content and qualitative data on the distribution of specific macro- and micro-elements in bean morphological regions without sample processing involving chemicals. No strong association was confirmed between bean elemental or physical profiles and production region. Nevertheless, the variation in elemental levels noted between beans typifies both bean nutritional and organoleptic qualities for specific regions. It should be noted that our elemental data are not directly comparable to previous reports describing the elemental content of organic (Barbosa et al., 2014) or conventional coffee (green, roasted, and instant) using conventional analysis (Zaidi et al., 2005; Filho et al., 2007; Grembecka et al., 2007; Liu et al., 2014; Habte et al., 2016; Hernández, Romero, Torres, Miranda, & Hernández-López, 2017) or PIXE (Debastiani et al., 2014; 2019) due to the factors described in the discussion influencing bean elemental profile. In terms of physical characterization, micro-CT scanning was particularly useful for the qualitative assessment of pore features and distribution in beans representing different geographical regions that may affect its shelf-life and mechanical properties.

In conclusion, this study showed the viability of a multidimensional approach employing external beam PIXE, light microscopy, and micro-CT scanning to provide physico-elemental data for screening the nutritional content and qualitative characteristics of coffee. In future, such type of multidimensional analysis may be particularly useful in understanding the chemical-microstructural-mechanical relationships to advise the development of models defining the ideal processing parameters to improve the quality of porous food products such as coffee (Oliveros et al., 2017).

Fig. 3. X-ray micro-computed tomography image of a coffee bean showing its microstructure in terms of porosity.

Fig. 4. Two-dimensional images of sagittal and transverse plane sections of roasted organic beans from Mexico (top row), Colombia (middle row), and Honduras (last row). Images in colour clearly indicate areas of high (red) or low (blue) pore interconnectivity, whilst grey-scale images reveal pore features. The smaller images show corresponding three-dimensional reconstructed images and the plane selected for 2D imaging. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
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.

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