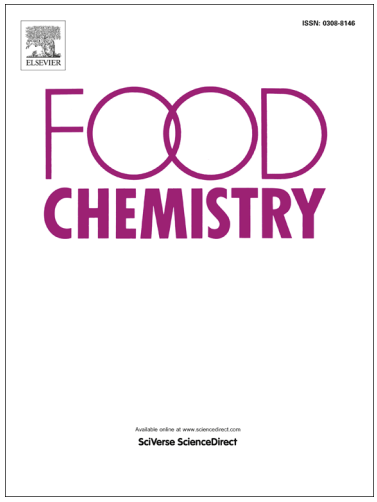

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Rapid multi-element characterization of microgreens via total-reflection X-ray fluorescence (TXRF) spectrometry

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ABSTRACT

Microgreens are an emerging class of vegetables, which have become increasingly important in the agri-food market in recent years, and contain a number of macro- and micro-nutrients. This paper presents a rapid method for the elemental analysis of microgreens based on total reflection X-ray fluorescence (TXRF) spectroscopy, without preliminary sample digestion.

The following elements were detected and quantified simultaneously for six microgreen genotypes, belonging to *Asteraceae* and *Brassicaceae*: P, S, K, Ca, Cl, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr. The limit of detection (LOD) varied depending on the element and ranged between 0.1 mg kg^{-1} for Sr and 42 mg kg^{-1} for P.

The method was validated using certified standards, and results compared with those obtained using a conventional ICP-AES method requiring sample digestion. The paper also presents the advantages and disadvantages of the two techniques.

Keywords

Microgreens, TXRF, ICP-AES, microelements, macroelements

1. Introduction

Microgreens are an emerging food consisting of young edible vegetables and herbs, which are harvested when cotyledonary leaves have fully developed and the first true leaves have emerged (usually 7-21 days after germination). The production of microgreens differs from sprouts and common freshly cut leafy vegetables, as microgreens are marketed together with their growing medium, which extends their shelf-life (Kyriacou et al., 2016). Recent studies have revealed that microgreens are richer than mature greens in some vitamins, sugars and antioxidants, including carotenoids (Kyriacou et al., 2019; Mir, Shah, & Mir, 2017; Sun et al., 2013; Xiao, Lester, Luo, & Wang, 2012; Xiao, Lester, Luo, Xie, Yu, & Wang, 2014; Xiao et al., 2019). Their consumption also appears to be associated with nutraceutical effects, i.e. a reduced risk of cardiovascular disease, possibly due to prevention of hypercholesterolemia (Huang et al., 2016), and also provides protection against inflammatory processes, oxidative stress and chronic diseases (Choe, Yu, & Wang, 2018). Few studies have investigated the mineral contents of microgreens, but these suggest that microgreens could provide an important supply of K, Ca, Fe and Zn (Pinto, Almeida, Aguiar, & Ferreira, 2015; Xiao, Codling, Luo, Nou, Lester, & Wang, 2016).

To date, multi-elemental characterization of microgreens has been carried out using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Xiao et al., 2016) and inductively coupled plasma mass spectroscopy (ICP-MS) (Pinto et al., 2015). Both methods require a complex and hazardous process of sample preparation based on acidic or alkaline digestion. These digestion

procedures often require special heating systems and apparatuses to prevent the loss of volatile elements, so that sample processing makes these analyses time-consuming and relatively expensive. Analytical techniques to perform elemental analysis without the need for sample digestion would be extremely useful to speed up sample preparation procedures, thereby reducing the cost of analysis and the risks involved in using chemicals.

X-ray fluorescence spectroscopy (XRF) is potentially a good alternative to ICP-AES, and usually requires very simple preparation of samples (e.g. fine grinding and pellet pressing). In conventional XRF, a primary X-ray beam is focused on the sample to expel electrons from the inner valence shells, causing the emission of secondary X-ray radiation, which is characteristic for each element in the sample. Both qualitative and quantitative elemental analysis can be performed according to the secondary X-ray beam energy (or wavelength) and intensity. Unfortunately, the high detection limits of conventional XRF (ranging from 10s to 100s mg kg^{-1}, depending on the element) make this technique less suitable for the elemental analysis of vegetables, in particular for micronutrients, whose concentrations usually range from 0.1 to 100 mg kg^{-1} dry weight.

It is possible to overcome the limits of conventional XRF by using a particular type of XRF, named total-reflection X-ray fluorescence spectroscopy (TXRF). In TXRF, the primary X-ray beam strikes the sample at an angle lower than the critical angle, making it possible to reduce sample self-absorption, thus increasing the signal-to-noise ratio and lowering detection limits compared with conventional XRF (Klockenkämper & von Bohlen, 2015). For this reason, TXRF has proven in recent years to be a useful and reliable analytical technique also for the analysis of trace elements in organic samples (Allegretta et al., 2017; De La Calle, Costas, Cabaleiro, Lavilla, & Bendicho, 2012; Stosnach, 2010), including vegetable foodstuffs (Dalipi, Borgese, Tsuji, Bontempi, & Depero, 2018; Dalipi, Marguí, Borgese, & Depero, 2017; De La Calle, Costas, Cabaleiro, Lavilla, & Bendicho, 2013). However, to the best of our knowledge, TXRF has never been used for the elemental analysis of microgreens.

In this paper, we propose a dedicated TXRF analytical method to study these innovative agricultural products, whose importance on the worldwide agrifood-market is expected to increase in the next few years. The proposed method has been developed on two certified reference standards and applied to six different genotypes of microgreens belonging to *Asteraceae* and *Brassicaceae*, analyzed using TXRF and ICP-AES, as the reference method. The results obtained with the two techniques were compared, and the advantages and disadvantages of the two techniques are presented below.

2. Materials and methods

2.1. Chemicals and standards

Nitric acid (≥69.0 %, TraceSELECT®), hydrogen peroxide (30%, TraceSELECT®), Triton™ X-100 and Ga standard solution (1000 mg L^{-1}, TraceCERT®) were purchased from Sigma Aldrich CHEMIE GmbH (Steinheim, Germany). The siliconizing solution (in isopropanol) was supplied by SERVA Electrophoresis GmbH (Heidelberg, Germany). Multi-element calibration standard (Certipur® ICP Multi-element standard solution IV, Merck GaA, Darmstadt, Germany) and phosphorous standard solution (Carlo Erba, Milano, Italy) were used for ICP-AES calibration. Double distilled deionized water was produced with a Milli-Q® system (Merck Millipore, Billerica, MA, USA). "Tomato leaves" (NIST 1573a) and "White cabbage" (BCR 690) certified reference materials were used for the validation of results.

2.2. Microgreens

Six different genotypes of microgreens were used in the experiments, four belonging to *Asteraceae* and two belonging to *Brassicaceae*. *Asteraceae* microgreens were: *Cichorium intybus* L. cv. 'Italico a costa rossa' (Chicory-1), *Cichorium intybus* L. var. 'Molfetta' (Chicory-2), *Lactuca sativa* L. Group *crispa* cv. 'Bionda da taglio' (Lettuce-1), and *Lactuca sativa* L. cv. 'Trocadero' (Lettuce-2). Both *Brassicaceae* microgreens belonged to *Brassica oleracea* L. Group *italic*; one was the local

variety 'Mugnuli' (Brassica-1), and the other cv. 'Natalino' (Brassica-2). Seeds for the four cultivars tested (Chicory-1, Lettuce-1, Lettuce-2 and Brassica-2) were purchased from Riccardo Larosa (Andria, Italy), while seeds for the two local varieties (Chicory-2 and Brassica-1) were obtained from local farmers. All the seeds were sown on a peat-based substrate (50% white-50% black peat mixture, Brill 3 Special, Brill Substrates, Georgsdorf, Germany) in plastic trays and kept in a growth chamber at 20°C at a relative humidity of 85%. Seeds germinated in the dark during the first two days and, then, irradiated (200 μmol m^{-2} s^{-1}) with a 12-h photoperiod. Microgreens were fertigated daily with a nutrient solution containing all the major and micro-nutrients at the following concentrations (mg L^{-1}): 105 N, 15 P, 117 K, 100 Ca, 24 Mg, 0.25 B, 0.01 Cu, 2.5 Fe, 0.25 Mn, 0.025 Zn, and 0.005 Mo. Microgreens were harvested after 12 days of growth using ceramic scissors to avoid metal contamination. For each genotype, approximately 100 g of plant material were sampled from three vessels. Samples were abundantly rinsed with tap water, then washed with bidistilled water, and freeze-dried (ScanVac CoolSafe 55-9 Pro; LaboGene ApS, Lynge, Denmark). The dried samples were stored in a desiccator until the analysis.

2.3. TXRF analyses

TXRF analyses were carried out on suspensions of certified reference materials and microgreens. Freeze-dried samples were pulverized with a vibro-milling system (MM 400, Retsch GmbH, Haan, Germany) operating at 30 Hz for 1 min, using zirconium oxide jars and balls. Then, 100 ± 5 mg of pulverized sample were weighted in a 12 mL-polypropylene tube with screw cap, and mixed with 5 mL of 1% Triton X-100 solution. An aliquot of 10 μL of Ga solution was added as internal standard, and the suspension was vortexed for 30 seconds. Triplicates were prepared and analyzed for each sample, including the certified materials. The suspensions were placed in an ultrasonic bath for 15 min, then vortexed for few seconds and an aliquot of 10 μL was pipetted on a siliconized quartz reflector and dried at 50°C on a hotplate. All the operations were carried out under a laminar flow hood.

Analysis were performed using a S2 Picofox TXRF spectrometer (Bruker Nano GmbH, Berlin, Germany), equipped with a Mo microfocus tube (30 W, 50 kV, 600 μA), a multilayer monochromator and a XFlash® silicon drift detector with 30 mm^2 active area. Energy resolution (Mn-Kα) was less than 150 eV (10 kcps). All the samples were analysed for 1000 s of live time. Deconvolution and analysis of TXRF spectra were performed using SPECTRA 7® software.

2.4. ICP-AES analyses

Sample preparation and digestion conditions followed the method described in Gattullo, Mininni, Parente, Montesano, Allegretta, and Terzano (2017). Briefly, an aliquot of 150 mg of powdered plant material was pre-digested overnight with 7 mL HNO_3 and 1 mL H_2O_2 in Teflon tubes at room temperature. Afterwards, samples were subjected to microwave-assisted digestion using a Multiwave GO (Anton Paar, Graz, Austria) system, following a single step heating programme (20 min to 180°C, held for 15 min, and final cooling). The same digestion procedure was adopted for the blank solution, which contained only 7 mL HNO_3 and 1 mL H_2O_2. The mineralized samples were diluted to 25 mL with deionised water, filtered with Whatman® 42 filter paper and stored at 4°C until analysis. Total concentrations of Na, Mg, P, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Sr, Cd and Pb were measured by inductively coupled plasma–atomic emission spectrometry (ICP-AES; Thermo iCAP 6000 series, Thermo Fisher Scientific Inc., Waltham, MA, USA). The emission wavelengths selected for the element quantification were (nm): Na 589.59, Mg 279.55, P 213.62, K 766.49, Ca 422.67, Cr 267.71, Cu 324.75, Mn 257.61, Fe 259.94, Ni 231.60, Zn 213.85, Sr 407.77, Cd 228.8, and Pb 220.35. The following plasma conditions were adopted: 1.15 kW RF power; 0.50 L min^{-1} auxiliary Ar flow; 0.90 L min^{-1} nebulizer Ar flow (0.5 L min^{-1} for Na, Mg, P, K, Ca and Sr determination); 12 L min^{-1} coolant Ar flow; axial plasma configuration. Each sample was analysed in triplicate. A two points calibration curve was employed, using i) the blank acidic solution as zero point and ii) a calibration solution prepared at the concentrations of 10 mg L^{-1} (for major elements) or 2 mg L^{-1} (for trace elements), by dilution with the blank acidic solution. Analysis of major

elements was carried out after diluting the samples with deionised water (1:10 v v^{-1} for Na, Mg and P, 1:50 v v^{-1} for K and Ca).

2.5 Parameters of validation

TXRF and ICP-AES results were validated determining the accuracy and precision. Accuracy was expressed as the recovery with respect to the certified concentrations of the reference materials, while precision was evaluated as the relative standard deviation (RSD). Recovery (expressed as %) was calculated as the ratio between the element concentration determined with ICP-AES or TXRF and the certified value, multiplied by 100. The RSD (%) was determined as the ratio between the standard deviation and the element concentration, multiplied by 100.

As for TXRF, the limits of detection (LOD) and quantification (LOQ) were calculated according to the following equations:

$$LOD_i = \frac{3C_i \cdot \sqrt{N_{BG}}}{N_i}$$

$$LOQ_i = \frac{10C_i \cdot \sqrt{N_{BG}}}{N_i}$$

where *Ci* is the concentration of the element *i*, *Ni* is the area of the fluorescence peak in counts, N_{BG} is the area of the background under the fluorescence peak (Klockenkämper & von Bohlen, 2015).

For ICP-AES, LODs and LOQs were calculated for each analyte as three and ten times, respectively, the standard deviation of ten replicates of the blank.

3. Results and discussion

3.1. Validation of the analytical method: TXRF vs ICP-AES analysis of standards

TXRF and ICP-AES results for "Tomato leaves" are reported in Table 1 and for "White cabbage" in Table 2.

TXRF identified the following elements in the "Tomato leaves" standard: P, S, Cl, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Br, Rb, and Sr. Recovery ranged between 91% and 111% for all elements except Ni,

for which recovery was 181% (Table 1). Overestimation of Ni might be caused by intense Ca pile-up peaks overlapping with Ni K-lines. It is likely that subtraction of Ca pile-up peaks by the software was insufficient to compensate for this effect, so that the Ni K-signal was overestimated. The LOD (and LOQ) decreased, moving from elements with a low atomic number (Z), such as P, towards elements with a higher Z, such as Sr. For Cd, the LOD calculated was higher (7.83 mg kg^{-1}), because the Mo X-ray source can excite only the Cd L-lines and not the more sensitive K-lines. In addition, Cd L-lines are situated in the same energy range as K and Ca K-lines, which are extremely intense in vegetable materials. The Cr Kα peak was also detected in the TXRF spectrum of "Tomato leaves". However, since the certified Cr concentration is lower than LOQ (Table 1), the concentration determined by TXRF (1.82 mg kg^{-1}) cannot be regarded as fully reliable, but may still be considered indicative. Excluding Cr, all the other elements were present at much higher concentrations than their LOQ. In particular, the concentration of minor elements (Mn, Fe, Ni, Cu, Zn, Rb, and Sr) was from 4 to 300 times higher than their LOQ.

The TXRF spectra of the "White cabbage" standard (Table 2) allowed identification of P, S, K, Ca, Mn, Fe, Ni, Cu, Zn and Sr. Recovery ranged between 85% and 109% for all elements except P (74%). This low P recovery might be explained by the shape of the "White cabbage" sample when the suspension is deposited on the quartz reflector. The deposition is concentrated in a spot with a diameter of approximately 2 mm and has a grainy structure with a small hump at the border, which may absorb a part of the fluorescence signal of light elements like P (Fig. S1, Supplementary material). An explanation for this behaviour might be that cabbage leaves are covered by large amounts of epicuticular waxes which, due to their hydrophobicity, tend to segregate on the quartz reflector after a drop of the sample is deposited. The same behaviour was not observed for "Tomato leaves" samples, which appeared more homogeneously distributed on a larger area at the centre of the quartz reflector (Fig. S1, Supplementary material). Phosphorous concentration was quantified more accurately for this standard.

ICP-AES results for the "Tomato leaves" standard (Table 1) ranged from 84% to 98% of recovery. The only exception was Mg, whose concentration was largely underestimated (70% recovery). Magnesium was also underestimated in the "White cabbage" standard (Table 2), where recovery was 76%. Nor was quantification of this element improved by changing the emission line from 279.55 to 280.27 or to 285.21nm. However, it must be said that Mg concentrations for both standards are only proposed and not certified. For all the other elements analysed in the "White cabbage" standard, recovery ranged between 81% and 106%. When the results obtained for the two standards were compared, elemental recovery was generally higher for "White cabbage", except for Cu and Ca. The reduced recovery rate in "Tomato leaves" standard might be explained by incomplete sample dissolution, which could be ascribed to the different Si concentrations in the two standard materials. In fact, Si can hinder total sample digestion when hydrofluoric acid is not used. According to Barros, de Souza, Schiavo, and Nóbrega (2015), Si concentration in "Tomato leaves" standard is 1800 mg kg^{-1}, while it is lower than LOD (56 mg kg^{-1}) in "White cabbage". Due to the low Si concentration, "White cabbage" was completely digested, as shown by the absence of any precipitate at the bottom of the digestion tube. Conversely, the digested solution of "Tomato leaves" appeared slightly turbid and a silica precipitate was visible at the bottom of the vessel. The LOD obtained for ICP-AES ranged between 2 and 8 mg kg^{-1} for Na, Mg, P, K, Ca, Fe, Ni and Zn, while for the other elements it was in the range 0.1-0.3 mg kg^{-1}. The lowest LOD was recorded for Cd (0.04 mg kg^{-1}).

One of the main differences between the TXRF and ICP-AES results concerns the type of detectable elements. TXRF did not detect Na and Mg because of the absorption of their fluorescence signals by atmospheric Ar, and because of the very low instrument sensitivity for these two elements. Detection of Na and Mg by TXRF may be possible working under vacuum and using X-ray sources whose target elements have lower atomic numbers, such as Cr (Hoefler, Streli, Wobrauschek, Óvári, & Záray, 2006; Streli, Wobrauschek, & Schraik, 2004), but these features are not currently available with commercial TXRF spectrometers.

Sulphur and halogens were not analysed by ICP-AES. Although quantification of these elements by ICP-AES is possible, accuracy may be significantly affected by the loss of volatile forms of S and halogens during digestion of the samples. Losses of volatile compounds may occur even in closed-vessel digestion systems, both during digestion (from the venting valves) and at the end of digestion (when opening the vessels), especially for samples containing large amounts of organic matter, which can undergo uncontrolled oxidative reactions. Another difference between TXRF and ICP-AES was the LOD of elements, which was comparable only for K, Ca, Cr, Mn, Cu and Sr. TXRF LOD for P and Cd were respectively 1 and over 2 orders of magnitude higher than those obtained by ICP-AES. This may not be a problem for the quantification of P (a major element in plants), but the high LOD of Cd is certainly a limiting factor for the technique. Together with Pb, Cd is one of the few elements whose concentration in vegetables is regulated by international laws (eg., European Commission Regulation, 2006). On the other hand, TXRF LODs for Fe, Ni and Zn are one order of magnitude lower than ICP-AES. These three elements are extremely important micronutrients since they are involved as enzyme cofactors in several biochemical processes (i.e. photosynthesis, respiration, N fixation, etc.) which are essential for the development of both plants and animals. Their detection is important for the assessment of plant nutritional status in eco-physiological studies (Mohamed, Rashed, & Mofty, 2003; Welch, 1995) and also for the evaluation of nutraceutical properties of vegetables. Independently of the type of certified material, TXRF gave more accurate and precise results for K and Ca than ICP-AES, whereas ICP-AES was more accurate and precise than TXRF for P quantification. As regards the other elements, the accuracy and precision of the results varied with the type of standard material. Considering the recovery obtained for both standards analysed using the two techniques, TXRF results appear closer to the certified values than ICP-AES results for “Tomato leaves”, while ICP-AES gave more accurate results for “White cabbage”. As stated before, this difference in behaviour might be ascribed to the sample preparation procedures; sample deposition of “White cabbage” on the quartz reflector was

imperfect when using TXRF, and sample digestion of "Tomato leaves" was incomplete when using ICP-AES. Both problems derived from the intrinsic characteristics of the matrix.

In conclusion, while both the techniques give similar results and in principle could be used interchangeably, one of the two may give more accurate results than the other for certain types of vegetables and would therefore be preferable.

3.2. Analyses of microgreens

Fig. 1 reports representative TXRF spectra of all the microgreens genotypes investigated, and Table 3 reports the results of elemental characterization of *Asteraceae* and *Brassicaceae* microgreens using both TXRF and ICP-AES. With the exception of Ni, elemental concentrations were far above the LOQ for all the microgreens analysed. Despite differences between the samples, K was the most abundant element in all the microgreens analyzed, with concentrations ranging between 38301 (Brassica-2) and 86760 mg kg^{-1} (Lettuce-2), depending on the genotype. *Asteraceae* microgreens presented higher K concentrations than *Brassicaceae*. Previous studies also confirm K as the main element accumulated in the tissues of lettuce (Pinto et al., 2015) and *Brassica* microgreens (Xiao et al., 2016). The second most abundant element was Ca, found in concentrations ranging between 15944 (Lettuce-1) and 33627 (Brassica-2) mg kg^{-1}, except for the two lettuce varieties, which were richer in Cl than in Ca. The levels of Mg measured in all the microgreens samples ranged from 200 mg kg^{-1} (Lettuce-1) to 252 mg kg^{-1} (Chicory-2) on a fresh weight basis, and were slightly lower than the values reported in the literature for lettuce and *Brassica* microgreens (Pinto et al., 2015; Xiao et al., 2016). However, Mg concentrations in the six genotypes were probably underestimated by ICP-AES, as discussed in Subsection 3.1. The levels of Na changed considerably with the plant genotype and were highest in Lettuce-1, with a value comparable to that reported by Pinto et al. (2015). Sulphur was particularly abundant in *Brassicaceae* microgreens, in concentrations approximately two to five times higher than those measured in *Asteraceae* microgreens. Of the two *B. oleracea* varieties, Brassica-2 presented the highest S content (26127 mg kg^{-1} *vs* 23292 mg kg^{-1} of

Brassica-1). This high S level is ascribable to the presence of S-containing secondary metabolites, which are typical of *Brassicaceae* plants, *i.e.* glucosinolates (Fahey, Zalcmann, & Talalay, 2001), and are very important in the human diet for cancer chemoprotection (Song & Thornalley, 2007). Regarding the micronutrients (Mn, Fe, Ni, Cu and Zn), Fe and Mn were the most abundant and the concentration of Mn was higher than that of Fe only in Lettuce-1 and Brassica-2. Nickel was the least abundant micronutrient, found at concentrations ranging between 1.5 (Brassica-2) and 4.6 (Chicory-1) mg kg^{-1}. Cadmium is a toxic element and was found in all samples, but in concentrations below the European legislation limit (European Commission Regulation, 2006). In particular, the maximum Cd concentration measured in microgreens (1.35 mg kg^{-1} in Lettuce-2 samples) was less than half the maximum admissible value set by the European regulations (200 µg kg^{-1} fresh weight, equivalent to 3.2 mg kg^{-1} dry weight, considering the mean dry weight of microgreen samples at 6%). The levels of Pb in all microgreen samples were always below the LOD of both TXRF (0.18 mg kg^{-1}) and ICP-AES (0.23 mg kg^{-1}), and consequently complied with the European legislation limit (300 µg kg^{-1} fresh weight, equivalent to 5 mg kg^{-1} dry weight; European Commission Regulation, 2006). Both Rb and Sr are non-essential elements for plants and have never been quantified in microgreens. Rubidium and Sr usually enter plant cells using, respectively, the transporters of K and Ca, because they possess similar properties and hydrated ionic radii (Marschner, 2012). Apart from Lettuce-2, all the other microgreens presented similar Rb concentrations (16 – 18 mg kg^{-1}). Strontium concentrations varied from a minimum of 55 mg kg^{-1} (Chicory-2 and Lettuce-1) to a maximum of 107 mg kg^{-1} (Brassica-2). The levels of Rb and Sr in microgreens fell within the range reported in the literature for vegetables (Kabata-Pendias & Mukherjee, 2007).

The element concentrations obtained for all the microgreen samples with the two techniques were compared using a regression line (Fig. 2). For elements such as Fe, Cu and Zn, R^2 ranged between 0.96 and 0.99, while the regression slope ranged from 0.99 to 1.13, showing close agreement between the data obtained using the two techniques. Data were more dispersed in the case of P and

K (R^2 = 0.88), but the correlation was still good. The slopes were 1.14 for P and 0.93 for K, which also showed a good agreement between TXRF and ICP-AES results. TXRF analyses gave generally higher concentrations than ICP-AES for Ca, Mn and Sr, with calculated slopes of 1.32, 1.26 and 1.27, respectively. The two sets of data were highly correlated (R^2=1.00) for Mn, while the correlation was lower for Ca (R^2=0.77) and Sr (R^2=0.85). In particular, the low correlation of Ca data might be ascribed to the digestion process. Calcium is mainly located in plant cell walls (Maathuis, 2009), which also contain Si. As said before, complete digestion of the sample may be affected by the presence of Si, which can be dissolved only by adding hydrofluoric acid during acid digestion (Feng, Wu, Wharmby, & Wittmeier, 1999), or by alkaline dissolution with NaOH after the acid attack (Barros et al., 2015). These treatments are more time-consuming and require additional consumption of dangerous chemicals. Moreover, the use of NaOH for alkaline mineralization may interfere with the quantification of Na in plant samples. Silicon is found at different concentrations in plants, according to their genotype and physiology (Barros et al., 2015), which means that the efficiency of the digestion might be different according to the species and variety. Chicory samples are known to contain less Si (200-500 mg kg^{-1}) than lettuce and broccoli samples (approximately 2000 and 1000 mg kg^{-1}, respectively) (D'Imperio, Renna, Cardinali, Buttaro, Santamaria, & Serio, 2016; D'Imperio et al., 2018; Ferreira Barreto, Schiavon Júnior, Maggio, & de Mello Prado, 2017; Galati, Marques, Morgado, Muniz, Filho, & Mattiuz, 2015), thus better sample dissolution of both chicory varieties can be hypothesized. TXRF and ICP-AES results for these two microgreens correspond more closely than for the others (Table 3). If the two chicory samples are not considered when calculating the Ca and Sr regression parameters, the correlation factor changes to 1.00 for Ca and 0.99 for Sr. Apart from K, the slopes of the regression lines are closer to the TXRF/ICP-AES ratios calculated for the "Tomato leaves" standard (Table 1) than to those calculated for "White cabbage" (Table 2). This strengthens the hypothesis that ICP-AES data might underestimate certain elements owing to incomplete sample digestion. Nickel concentrations measured by TXRF and ICP-AES cannot be compared, since ICP-AES results for Lettuce-1,

Lettuce-2 and Brassica-2 were lower than the LOD. Moreover, Ni concentrations were also lower than the LOQ of ICP-AES (8.8 mg kg^{-1}) for the other microgreens. However, Ni was better quantified by TXRF in all microgreens (LOQ= 0.67 mg kg^{-1}). In this case, Ca pile-up lines were not a problem, since Ca concentration in microgreens was not as high as in "Tomato leaves" and did not significantly interfere with the Ni K-line spectral region.

4. Conclusions

TXRF allows simultaneous detection and quantification of 4 macro- (P, S, K, Ca), 6 micro- (Cl, Mn, Fe, Ni, Cu, Zn) and 3 non-essential (Br, Rb, Sr) elements after simple and rapid sample preparation which does not require a preliminary dissolution step. This reduces the time, cost and environmental impact of analysis. In addition, volatile elements are not lost, and problems related to incomplete sample digestion (especially important for Si-rich vegetables) are overcome. Moreover, TXRF gave a lower detection limit for Ni than ICP-AES, allowing the quantification of this element also at concentrations lower than 10 mg kg^{-1} dry weight. However, unlike ICP-AES, TXRF cannot analyse Na or Mg, due to atmospheric Ar absorption of the K-lines of these elements. Development of new commercial TXRF instruments working under vacuum or He atmosphere may help to overcome this limitation in the future. The most serious drawback is that TXRF is much less sensitive than ICP-AES for Cd detection, which is extremely important for the food-safety assessment of vegetables.

In general, TXRF is a valid alternative to ICP-AES for the simultaneous multielemental analysis of microgreens, and involves simpler, cheaper and faster sample preparation and lower instrumental operational costs. Furthermore, TXRF instruments are also compact and robust and can be transported easily for on-site measurements. The advantages and disadvantages of the two techniques for the analysis of microgreens are summarized in Table 4.

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Declarations of interest

None

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at …

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Figure captions

Fig. 1 TXRF spectra of the six genotypes of microgreens. From the bottom: *C. intybus* cv. 'Italico a costa rossa' (Chicory-1); *C. intybus* var. 'Molfetta' (Chicory-2); *L. sativa* cv. 'Bionda da taglio'

(Lettuce-1); *L. sativa* cv. 'Trocadero' (Lettuce-2); *B. oleracea* var. 'Mugnuli' (Brassica-1); *B. oleracea* cv. 'Natalino' (Brassica-2).

Fig. 2 Correlations between ICP-AES and TXRF results for P, K, Ca, Mn, Fe, Cu, Zn and Sr measured in the six genotypes of microgreens.

Fig. S1 Microscopic images (10x) and magnifications (100x) of "Tomato leaves" and "White cabbage" depositions on quartz reflectors.

Table 1 Elemental composition (mg kg^{-1} dry weight) of NIST 1573a standard reference material ("Tomato leaves") measured by TXRF and ICP-AES (n=3).

Element	Certified value		TXRF suspension							
	Concentration	SD[a]	Concentration	SD	LOD[b]	LOQ[c]	Recovery	RSD[d]	Concentration	SD
	mg kg^{-1}		mg kg^{-1}				%		mg kg	
Na	136	4	-	-	-	-	-	-	130	
Mg	12000[e]	-	-	-	-	-	-	-	8365	
P	2160	40	2406	113	42	140	111	5	2126	9
S	9600[e]	-	10167	466	25	83	106	5	-	
Cl	6600[e]	-	5995	334	13	43	91	6	-	
K	27000	500	26507	1097	5	17	98	4	23058	12
Ca	50500	900	53726	2398	2	7	106	4	44383	30
Cr	1.99	0.06	(1.82)[f]	0.02	0.63	2.10	91	1	1.94	0.
Mn	246	8	260	12	0.5	1.7	106	5	211	
Fe	368	7	363	14	0.4	1.3	99	4	309	
Ni	1.59	0.07	2.89	0.11	0.20	0.67	181	4	<LOD	
Cu	4.70	0.14	4.39	0.27	0.20	0.67	93	6	4.40	0.
Zn	30.9	0.7	29.2	1.3	0.2	0.7	94	7	30.1	1
Br	1300[e]	-	1195	45	0.2	0.7	92	4	-	
Rb	14.89	0.27	14.72	0.27	0.15	0.50	99	2	-	
Sr	85[e]	-	85	4	0.1	0.3	100	5	71	
Cd	1.52	0.04	<LOD	-	7.83	26.10	-	-	1.47	0.

[a] Standard deviation
[b] Limit of detection
[c] Limit of quantification
[d] Relative standard deviation
[e] Proposed value
[f] Lower than LOQ (indicative value)

Table 2 Elemental composition (mg kg^{-1} dry weight) of BCR 679 standard reference material ("White cabbage") measured by TXRF and ICP-AES (n=3).

Element	Certified value	TXRF suspension

hicory-1[a]			Chicory-2				Lettuce-1				Lettuce-2				Brassica-1				B	
D[b]	ICP-AES	SD	TXRF	SD	ICP-AES	SD	TXRF	SD	ICP-AES	SD	TXRF	SD	ICP-AES	SD	TXRF	SD	ICP-AES	SD	TXRF	SD
	mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}	
-	1257	164	-	-	1649	465	-	-	9112	288	-	-	1332	232	-	-	3349	97	-	
-	3752	271	-	-	3954	403	-	-	3849	314	-	-	4547	144	-	-	3062	41	-	
96	11341	371	14834	700	11957	323	10288	555	8209	456	15980	1368	13125	252	10128	3034	7213	141	13152	2
25	-	-	9240	1123	-	-	4754	211	-	-	12279	83	-	-	23292	4875	-	-	26127	3
87	-	-	13419	1737	-	-	28231	1545	-	-	35740	3342	-	-	9515	1681	-	-	20969	6
44	57508	3173	58689	4106	54916	4157	55024	2414	54661	3748	86760	9639	84966	2606	42958	10715	42909	9917	48451	8
92	23681	1111	19989	1315	19947	2169	19367	958	15944	1130	26863	162	21887	696	27716	1907	22347	1133	33627	3
14	82	6	117	15	99	6	254	11	202	13	121	1	96	1	78	13	64	8	133	
6	172	11	221	44	210	16	82	3	74	2	305	14	261	6	120	42	109	40	97	
0.3	3.6	0.8	4.1	0.2	2.9	1.2	1.9	0.2	<2.63		2.8	0.5	<2.63		2.0	1.3	2.8	0.2	1.5	0
0.1	18	0.3	13	1	14	0.2	8.7	0.4	8.9	0.2	16	2	16	0.1	4.8	1.0	4.3	1.3	6.4	0
2	72	3	69	5	75	7	90	2	90	3	96	3	97	1	53	14	54	18	77	
3	-	-	15	3	-	-	47	2	-	-	33	1	-	-	20	4	-	-	44	
0.2	-	-	18	2	-	-	18	1	-	-	27	3	-	-	16	1	-	-	16	0
4	84	3	60	5	55	2	63	3	55	3	97	1	77	1	91	6	76	4	107	
-	0.55	0.05	-	-	0.69	0.04	-	-	1.18	0.07	-	-	1.35	0.05	-	-	0.28	0.01	-	

	Concentration	SD[a]	Concentration	SD	LOD[b]	LOQ[c]	Recovery	RSD[d]	Concentration	SD
	mg kg^{-1}		mg kg^{-1}				%		mg kg	
Na	n.d.[e]	-	-	-	-		-	-	930	6
Mg	1362[f]	127	-	-	-		-	-	1035	1
P	3307[f]	241	2451	200	30	100	74	8	3340	11
S	n.d.	-	7453	518	19	63	-	7	-	
K	n.d.	-	24780	1761	6	20	-	7	25532	196
Ca	7768[f]	655	7692	180	2	7	99	2	6831	63
Mn	13.3	0.5	14.5	0.4	0.4	1.3	109	3	12.3	0
Fe	55	2.5	48	8	0.3	1.0	87	17	57	
Ni	27	0.8	27	0.4	0.2	0.7	100	1	29	
Cu	2.89	0.12	2.47	0.06	0.18	0.60	85	2	2.35	0.1
Zn	79.7	2.7	76.9	0.9	0.2	0.7	96	1	80.9	2
Sr	11.8	0.4	10.1	0.1	0.2	0.7	86	1	10.9	0
Cd	1.66	0.07	<LOD	-	5.51	18.37	-	-	1.67	0.0

[a] Standard deviation
[b] Limit of detection
[c] Limit of quantification
[d] Relative standard deviation
[e] Not determined
[f] Proposed value

Table 3 Comparison between TXRF and ICP-AES results of elemental characterization of six different genotypes of microgreens. Mean values of concentrations are expressed on a dry weight basis.

[a] Microgreen genotypes: *C. intybus* cv. 'Italico a costa rossa' (Chicory-1); *C. intybus* var. 'Molfetta' (Chicory-2); *L. sativa* cv. 'Bionda da taglio' (Lettuce-1); *L. sativa* cv. 'Trocadero' (Lettuce-2); *B. oleracea* var. 'Mugnuli' (Brassica-1); *B. oleracea* cv. 'Natalino' (Brassica-2)

[b] Standard deviation (n=3)

Analytical method	Advantages	Disadvantages
TXRF	No sample digestion	Necessity of appropriate milling systems to pulverize the sample very finely (< 50 μm)
	No necessity of microwave heating systems	Impossibility to quantify Na and Mg, unless analyzing the sample under vacuum or He atmosphere
	Instrument calibration with a single element standard solution	High detection limit for Cd
	No dilution of the sample before the analysis	Imperfect deposition of materials characterized by high content of hydrophobic compounds like waxes can bias the quantification of light elements
	Simultaneous determination of all the elements from P to Pb	Lower accuracy and precision for P determination
	No limitations for the analysis of Si-rich materials	
	Low detection limit for Fe, Ni and Zn	
ICP-AES	Quantification of Na and Mg is possible	Sample digestion needed
	Very low detection limit for Cd	Underestimation of some elements in Si-rich samples due to incomplete sample dissolution
	Higher accuracy and precision for P determination	Loss of volatile elements like S and halogens
		Need of (microwave) heating system for sample digestion
		Instrument calibration with a standard solution containing all the elements to be analyzed
		Preparation of different dilutions of the sample depending on the type of elements to be analyzed (i.e., major or trace elements)
		Simultaneous determination only for elements in the same concentration range

Table 4 Summary of the advantages and disadvantages of TXRF and ICP-AES methods for the analysis of microgreens.

Highlights

Microgreens are a relevant source of mineral macro and micronutrients

Microgreens can be analysed by TXRF without sample digestion

Simultaneous multi-elemental analysis can be performed by TXRF

TXRF detection limits vary from 0.1 to 42 mg kg^{-1}, depending on the element being analysed

The method is cheaper and faster than ICP-AES for most elements