ARTICLE

Total reflection X-ray fluorescence technique for multi-elemental analysis of food

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Introduction

Elemental analysis is needed more and more in the food industry; from a nutritional point of view and for safety and quality purposes. Food is our most significant source of major (Ca, Cl, C, H, Mg, N, O, P, K, Na, S), minor-trace (F, I, Fe, Si, Zn) and ultra-trace (Cr, Co, Cu, Mn, Mo, Ni, Se, V) essential elements. However, non-essential and/or potentially toxic trace elements, like Al, As, Cd, Hg, Pb, Sb and U, may also contaminate food, entering the food chain from the environment, processing and storage.

Spectroscopic techniques like inductively coupled plasma mass or atomic emission spectrometry (ICP-MS or ICP-AES) are usually selected for elemental determination in foodstuffs, due to their multi-elemental capabilities and low limits of detection. These techniques require some additional sample treatment for the total destruction of organic matrices: mainly acidic digestion, especially for solid samples. This is a critical step involving the use of dangerous reagents. Moreover, losses of analytes by volatilisation are common, and the procedure itself is very time-consuming. The use of other methodologies, including X-ray fluorescence (XRF) spectrometry, for direct analysis of solid food samples has increased over the last few years. Among XRF techniques, total reflection X-ray fluorescence (TXRF) is preferred, having higher sensitivity and a limit of detection at the nanogram level.

Basic principles and analytical capabilities of TXRF

TXRF is a variation of energy dispersive XRF spectrometry (EDXRF). It differs from EDXRF mainly in the experimental setup geometry. An angle lower than 0.1° is required in TXRF in order to obtain the reflection of the whole beam on the reflector, instead of the conventional angle of 45°, as illustrated in Figure 1.

TXRF is primarily used for chemical micro and trace analyses. For these purposes, small quantities, mostly solutions and suspensions, are deposited on optical flat reflectors, i.e. quartz glass, and evaporated to dryness. After that, the residue is measured in a spectrometer equipped with energy dispersive detector positioned a few millimetres above the reflector surface. Most of the incident beam radiation is reflected and, as a consequence, the spectral background is reduced. In this way, the fluorescence yield is very high and absorption effects minimised. These characteristics allow better detection limits (10^{-7} to 10^{-12} g) compared to those of conventional EDXRF. Matrix effects are negligible if the thin film requirements are fulfilled, i.e. samples that are thinner than the critical thickness.

Several TXRF spectrometers are commercially available. The main producer companies are Bruker (S2 Picofox, S4 T-Star), Rigaku (Nanohunter), GNR (TX 2000) and
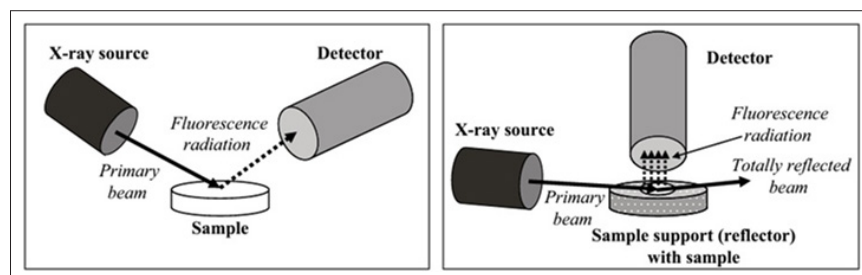

Figure 1. Instrumental setup for conventional XRF (left) and TXRF (right).

Table 1. Instrumental parameters of TXRF spectrometers.

S2 PICOFOX TXRF benchtop spectrometers		
Anode	**Mo**	**W**
X-ray tube	Air-cooled metal ceramic	Air-cooled metal ceramic
Maximum power	40 W	50 W
Optics	Multilayer monochromator (17.5 keV)	Multilayer monochromator (35 keV)
Detector	Silicon drift detector, Area: 30 mm^2, FWHM: 139.43 eV (Mn Kα)	Silicon drift detector, Area: 10 mm^2, FWHM: 146.72 eV (Mn Kα)
Filter	Mo 10.00 μm	Ni 50.00 μm
Sample changer	Manual version for single samples	Automatic version with cassette for up to 25 samples
Atmosphere	Air	Air
Voltage	50 kV	50 kV
Current	750 μA	1000 μA
Live time	600 s	2000 s

ATI (Wobistrax). The most recent TXRF spectrometers are benchtop instruments equipped with low-power, air-cooled X-ray tubes.

In the next sections several analytical TXRF methodologies for the analysis of solid food samples are described. In all cases, benchtop systems have been used (Bruker S2 Picofox TXRF spectrometer). Table 1 shows the instrumental setups and measurement parameters.

Application of TXRF in foodstuff analysis

The first paper about elemental analysis of foodstuff by means of TXRF dates back to 1989. The number of publications in this field has increased during the last ten years. Today, TXRF is emerging as a powerful tool for food analysis, especially where a holistic approach is followed. Drinks, beverages, vegetables, fruits, herbs, spices, cereals, animal derivatives and dietary supplements as foodstuff samples have been analysed by TXRF for safety and quality purposes. Figure 2 shows the trend of publications related to TXRF analysis of foodstuff and the percentages of the fields of application. In this paper we will focus on some examples of TXRF analysis of seafood, vegetables and honey, considering sample preparation procedures and methods developed to obtain accurate and reliable results.

More details can be found in the literature.[1–3]

Seafood analysis

It is well known that bivalves may accumulate large amounts of metals and for this reason they are widely used as bio indicators, but they are also analysed for food safety purposes. Here we show the analysis of different commercial clam species from Portuguese markets for the determination of elements in the high–medium mg kg^{-1} range (higher than 5–10 mg kg^{-1}), comparing two different sample preparation procedures.[1] For this purpose, the soft tissues were freeze dried and ground to a particle size less than 100 μm. The conventional microwave digestion with a mixture of $HNO_3 + H_2O_2$ and sample suspension in a disperser solution were compared as sample treatments for TXRF analysis. Different amounts (20, 50 and 100 mg) and dispersant solutions (ultrapure water and 0.1% Triton® X-114) for the preparation of suspensions were tested. For quantification, Y was used as internal standard. All the tests were performed using the reference material GBW08571 "mussel muscle tissue". Measurements were performed with W excitation.
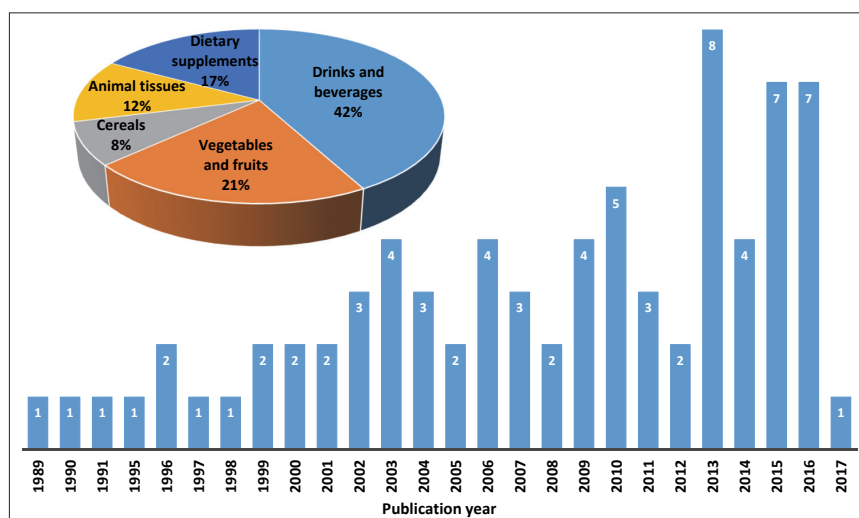

Figure 2. Number of publications related to TXRF analysis of foodstuff and the fields of application (source ISI Web of Knowledge).
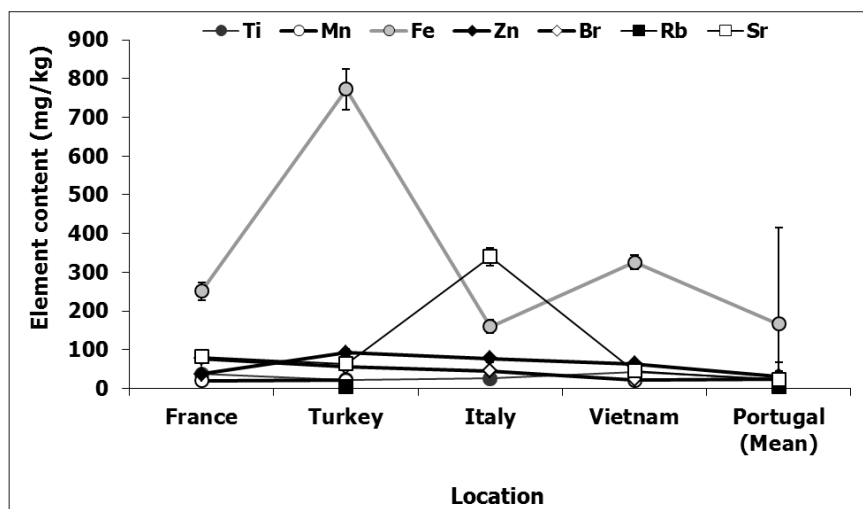
ARTICLE

Figure 3. Minor elemental determination in the studied edible clams from different locations.

It was found that the best conditions for the analysis of solid biological samples are 100 mg of sample and 1 mL of ultrapure water as disperser agent. The comparison between acid digestion and suspension showed comparable results. The results of the analysis of commercial edible clams from different locations by means of TXRF are shown in Figure 3. As we can see, similar concentrations were obtained for all the locations, except for Fe in Turkish and Sr in Italian clams. The measured concentration levels agree with literature data.

Honey analysis

Elemental determination in sugar-rich foodstuff samples has been a challenging analytical task for researchers due to matrix effects. Among all such foods, honey is the most studied, both for environmental and food safety reasons. In this work, we show a quick and simple analytical method for multi-elemental analysis of different honey samples by means of a TXRF system equipped with a Mo anode X-ray tube.[2] For this purpose, 45 honey samples with different botanical and geographical origins were selected. Each sample solution was prepared by mixing about 0.5 g of honey and 10 mL of ultrapure water. The volume of 1 mL of each sample solution was added with Ga and thoroughly homogenised, to prepare the specimen for

TXRF analysis with a final Ga concentration of 0.5 mg L^{-1}. Three replicates were prepared depositing 10 µL of specimen on each quartz glass sample carrier and dried.

Our data are in agreement with those reported in the literature for similar studies and different spectrometric techniques, such as AAS and ICP-MS. All these data were used together for chemometric investigation. Single botanical origin honey samples were considered. Principal component analysis (PCA) allowed a clear differentiation according to botanical origin (Figure 4), where K, Mn, Zn and P give the main contribution. These results clearly show that TXRF

provides results comparable to the other techniques.

Vegetal foodstuff analysis

Vegetables are of fundamental importance in the human diet and are primary recipients of essential and potentially toxic elements from the environment. These elements are transferred to humans directly through eating, or indirectly through milk and meat from animals fed with vegetables. In this work we demonstrate that TXRF allows accurate and precise analysis of lyophilised vegetables, with a simple and fast procedure for sample preparation. Six certified reference materials provided by the National Institute of Standards & Technology (NIST) were selected: SRM 1515 (apple leaves), SRM 1547 (peach leaves), SRM 1570A (spinach leaves), SRM 1572 (citrus leaves), SRM 1567A (wheat flour) and SRM 1568A (rice flour). The solid samples were prepared by suspending about 20 mg of powder in 1 mL of dispersant solution (ultrapure water or 1% Triton X-100). Gallium was added as internal standard with a final concentration of 10 mg L^{-1}. Duplicates were prepared for each sample and 10 µL were deposited on a siliconised quartz reflector and dried. Measurements were performed with two low-power benchtop TXRF systems equipped with Mo and W X-ray tubes.[3]
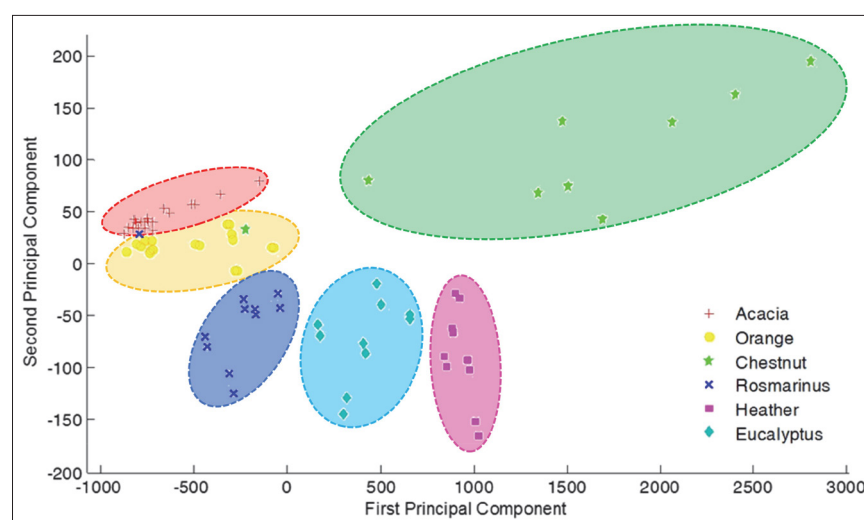

Figure 4. PCA Scatter plots of TXRF and other spectroscopic techniques data found in the literature with different botanical and geographical origins.
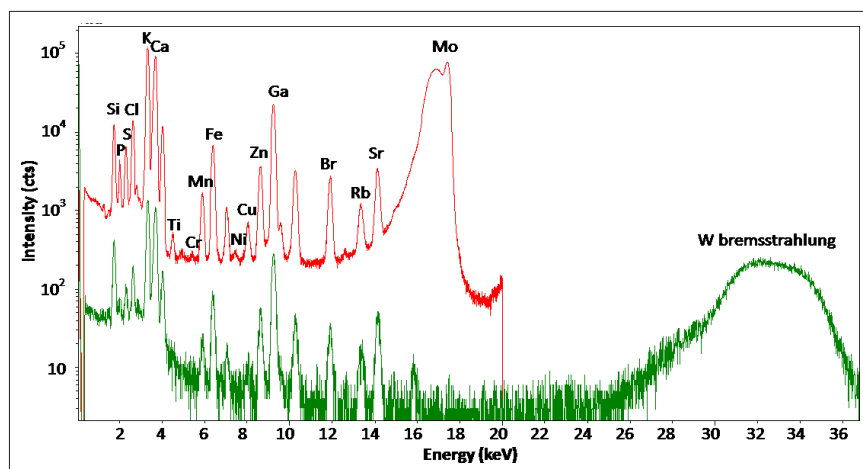
Figure 5. TXRF raw spectra for the analysis of SRM 1570A sample obtained using Mo (red spectrum) and W (green spectrum) as excitation sources.

The typical raw spectra of SRM 1570A are shown in Figure 5. The main differences between Mo and W excitation are in the measurement energy (keV) range and the background produced. The Mo TXRF system has better sensitivity and detection limits are lower compared to the W system, with some exceptions. Indeed, the W system is more suitable for the determination of high Z elements like Cd and Hg that cannot be determined with the other system.

Results are summarised in Figure 6 and compared with certified values. Most of the values are in agreement.

Differences for light Z elements like K and Ca are due to the absorption effects when measurements are performed in air (not under vacuum conditions). Moreover, due to the limited sensitivity of the W TXRF system, elements in very low concentrations could not be determined.

Concluding remarks and future perspectives

In this work, we have shown the suitability of low-power, benchtop TXRF instrumentations equipped with Mo and W X-ray tubes for multi-elemental analysis of different foodstuff samples. TXRF offers a fast and simple way to perform screening and reliable quantitative analysis of food samples with complex matrices. TXRF may be successfully used for food safety, traceability and quality control. Moreover, TXRF has some advantages over other spectroscopic techniques, such as the possibility to get simultaneous multi-elemental information, the low amount of sample required to perform the analysis and the possibility to get quantitative results without external calibration. The use of TXRF is still limited due to the lack of recognised standard methods, and overcoming this will require a significant collaborative effort to develop guidelines for experimental procedures. Future improvements in TXRF devices and sample pre-treatments are expected to offer further approaches for low-cost routine and on-line analysis.

Figure 6. Comparison of the results obtained by Mo and W TXRF systems and certified values. Error bars represent the standard deviation of duplicates.

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3. R. Dalipi, E. Marguí, L. Borgese and L.E. Depero, "Multi-element analysis of vegetal foodstuff by means of low power total reflection X-ray fluorescence (TXRF) spectromet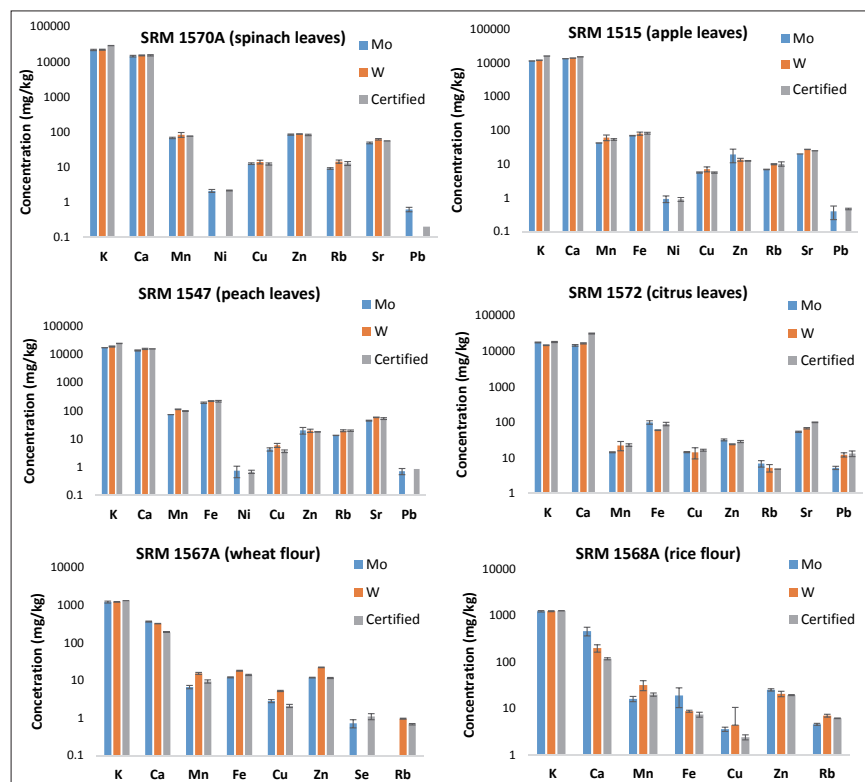ry", *Food Chem.* **218,** 348 (2017). doi: https://doi.org/10.1016/j.foodchem.2016.09.022