

## TOTAL REFLECTION X-RAY FLUORESCENCE AS A TOOL FOR FOOD SCREENING

Fabiola Bilo<sup>1</sup>, Laura Borgese<sup>1,2</sup>, Annalisa Zacco<sup>1,2</sup>, Rogerta Dalipi<sup>1</sup>, Elza Bontempi<sup>1,2</sup>,  
Laura E. Depero<sup>1,2</sup>

<sup>1</sup> Chemistry for Technologies Laboratory, Department of Mechanical and Industrial Engineering,  
University of Brescia, Brescia, Italy,  
email: f.bilo@unibs.it

<sup>2</sup> SMART Solutions s.r.l, Brescia, Italy

**Abstract** – Elemental composition of food is of great importance, since food is the main source of essential, major and trace elements for humans. Some potentially toxic elements may contaminate food, entering the food chain from the environment, processing, and storage. Therefore, the elemental analysis of food is fundamental for safety assessment. Fast and sensitive analytical techniques are required as a result of the increasing demand on multi-elemental analysis and product screening. Total reflection X-Ray Fluorescence (TXRF) is suitable technique, since it provides simultaneous multi-elemental identification in a wide dynamic range of concentrations. In this review, several different matrices such as drinks, beverages, vegetables, fruits, cereals, animal derivatives and dietary supplements are analysed by TXRF for food quality monitoring. The obtained results reveal a good precision and accuracy, highlighting TXRF as a competitive technique with atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS).

**Keywords:** Heavy Metals, Food Quality and Safety, Direct Analysis, TXRF, Traceability, Screening

### 1. INTRODUCTION

Food security is a growing global issue because human health is strongly dependent on its quality. Therefore, it should guarantee the absence of contaminants and the presence of nutrients. World Health Organisation (WHO) reports the maximum accepted guideline limits for heavy metals concentration in foodstuff. Elements like K, Ca, Mg, P, Na are considered as indispensable for growth and health, while microelements (Mn, Fe, Cu, Zn, Se, Mo) are essential for human nutrition, but

excessive quantities may be toxic [1]. Other elements, like Cd, As, Hg and Pb, are toxic even in trace and thus must not enter in the food chain [2]. In this frame, elemental chemical analysis needs to be applied at different steps of production and storage process in order to ensure the food safety.

Multi-elemental chemical analysis of foodstuff samples is a difficult process, due to the contemporary presence of organic and inorganic substances. For this reason, most of the analytical techniques for heavy metals analysis require sample pre-treatment procedures such as microwave acid digestion [3], dry ashing [4] wet digestion [5]. The elemental determination is performed by atomic absorption spectroscopy (AAS) [6], and inductively coupled plasma mass spectrometry (ICP-MS) [7].

Nowadays, there is a great interest on analytical methods which offers a multi-elemental information, less or without pre-treatment sample preparation, lower limit of quantification, higher repeatability, reproducibility, and recovery. Total reflection X-Ray Fluorescence (TXRF) spectroscopy fulfils the above mentioned requirements and it is already proved as a suitable technique for food screening [8].

Furthermore, TXRF offers some advantages with respect to other spectroscopic techniques such as low amount of sample to perform the analysis (few  $\mu\text{L}$  or ng), fast and easier quantification by internal standardization and no matrix effects [9, 10]. Besides, new low power benchtop TXRF systems are really cost-effective since they do not require gas or cooling media.

The aim of this study is to demonstrate the successful applications of TXRF in food quality monitoring and provide a critical outlook on the developments and improvements required to transfer these methods from research to the industrial and analytical laboratories contexts.

## 2. FIELDS OF APPLICATIONS

In this study TXRF application for food analysis, comprising drinks and beverages, is considered. Figure 1 shows TXRF field of application and their percentages, respectively.

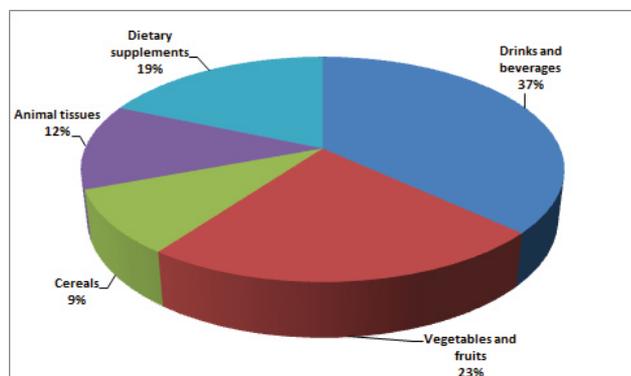


Fig. 1. Percentage of the foodstuff classes analysed by TXRF technique.

In Table 1 are reported the food matrixes and sample preparation procedure followed for TXRF analysis.

Table 1. Foodstuff samples analysed by TXRF.

Food Matrix	Samples	Sample Preparation
Drinks & Beverages	Drinking water	Direct Analysis
	Wine	Direct Analysis
	Alcoholic beverages	Dry Ashing
	Soft beverages	Direct Analysis
	Milk	Acid Digestion
	Coffee	Direct Analysis
Vegetables & Fruits & Herbs & Spices	Tea	Direct/Infusion
	Cauliflower, onions, cucumber, lettuce	Acid Digestion
Fruits & Herbs & Spices	Pineapple, apple	Ultrasound Extraction
	Medical Plants, Herbs.	Direct Analysis
Cereals	Herbs.	Ultrasound Extraction
	Rice	Suspension
	Wheat Flour	Acid Digestion
Animal Tissues	Porridge Flour	Direct Analysis
	Fish Tissue	Wet Ashing
	Mussels	Acid Digestion
Dietary Supplements	Hen eggs	Direct Analysis
	Honey	Wet Ashing
	Pollen	Acid Digestion
	Bee pollen & Tissue	Direct Analysis
	Candies	Acid Digestion
	Chocolate	Acid Digestion

### 2.1. Drinks and Beverages

Successful applications of TXRF in drinking water, milk, wine, tea, soft and alcoholic drinks are already reported. Indeed, drinks and beverages numbered

the highest percentage of TXRF analysis (37%) and are classified as the easiest sample because of the fast and simple preparation. Direct analysis of mineral water is strongly recommended. In case of very low element concentration, suitable pre-concentration and microextraction methodologies have been developed. Precipitation of Chlorine as AgCl was proposed as a suitable method for total Cl determination [11], while perchlorate analysis was achieved through application of selective membrane on the sample carrier surface [12].

### 2.2. Vegetables, fruits, herbs and spices

In literature are reported successful application of TXRF for the food quality control of fruits, vegetable leaves, vegetable roots, herbs and spices. Thanks to high sensitivity of the technique, even trace quantities of toxic metals were detected. Acid digestion, suspension and extraction are considered as the most useful sample pre-treatment methods. The mineralization is reported as the preferred method, giving the best results in terms of reproducibility, accuracy, and precision. On the other hand, the suspension is faster and simpler, avoiding contamination and loss of material. However, sample homogenization is critical and high background may be present. Comparison of TXRF data with those obtained by other spectroscopic techniques like FAAS, flow injection atomic absorption spectroscopy (FIAAS) and hydride generation atomic absorption spectrometry (HG-AAS) reveals that TXRF may be a reference technique for the elemental analysis.

Statistical test such as Principal Component Analysis (PCA) or Linear Discriminant Analysis (LDA) are successfully applied. LDA was used to distinguish the anatomical part of herbs: flower, leaf and fruit [9].

### 2.3. Cereals

Cereals are the most important and essential foodstuffs for human diet. Although TXRF analysis of cereals is not so common, studies have developed methodologies for analysis of rice, wheat and porridge flour. The achieved detection limits for trace elements are suitable for the precise and accurate determination in cereals. Cluster analysis was applied to distinguish brown from white rice. It was found that brown rice contains higher content of Cu, Zn and the lower concentration of Ca, Fe.

Based on the reported data, the analysed samples respect the WHO guideline limit concentrations for all the essential nutrients, ensuring the food safety [13].

#### 2.4. Animal Tissues

Although, fish and other animal tissues are known as rich products with proteins and nutrients, they may accumulate toxic elements, depending on the environment conditions and dietary intake [14]. Meanwhile, these organisms may be used as indicator of environmental pollution. Acid/wet digestion and direct analysis were the suggested procedures for TXRF analysis. Both the proposed sample preparation procedures gave precise and accurate results, allowing the determination of metals accumulation. A correlation between the tropic levels of system and metal concentration was observed. Indeed, higher metals content was met in the highest tropic level. Results of different studies highlighted that most of the studied samples exceeding the maximum tolerance levels and muscle was the target organ [15, 16, 17]. Elemental chemical analysis of eggs samples coming from poultry farm and supermarket was performed after total mineralization. It was found a correlation between the element concentration in eggs and seasonality. Macronutrients concentration was higher in egg albumen and yolk samples produced in the winter season, while the highest amount of micronutrients was detected in egg yolk samples collected in summer.

#### 2.5. Dietary Supplements and others

Dietary supplements are defined as products that increase the total intake of minerals, amino acids, vitamins. Honey, pollen, candies and different dietary supplements for I and Se determination are considered.

Honey studies deal with multi-elemental determinations in bee honey, pollen and bee tissue to obtain useful information about the environment conditions and the variety of honeys. Comparison between TXRF and AAS or ICP-AES results figured out the advantages of TXRF for simultaneous multi-element analysis. Moreover, the elemental analysis of honey by means of TXRF is a good source of data for chemometric statistical tests, with aim to distinguish between the botanical and geographical origin. In Figure 2 is shown PCA performed for

honey samples. Six groups can be clearly distinguished, corresponding to the six botanical origins of the honey. Main contributing elements are K and Mn for the first principal component, Zn and P for the second principal component.

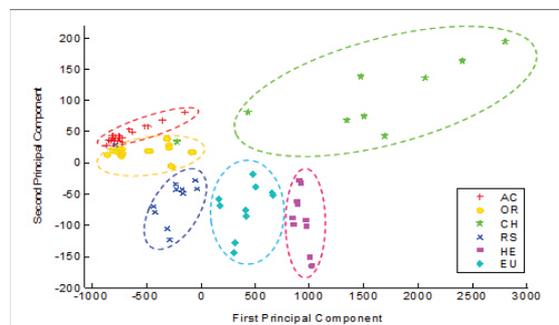


Fig. 2. PCA Scatter plots of TXRF and other spectroscopic techniques data found in the literature with different botanical and geographical origins. AC: acacia, OR: orange, CH: chestnut, RS: rosmarinus, HE: heather, EU: eucalyptus.

Great attention is focused on supplements consumed by children like candies and chocolate. TXRF analysis was performed after mineralization in a mixture of acids such as  $\text{HNO}_3 + \text{HCl} + \text{H}_2\text{O}_2$ . Results of candies samples revealed higher concentration of Pb than the recommended maximum accepted limit [18], while Ni and Br amount were lower than the guideline levels.

TXRF analysis was successfully applied in other dietary supplements samples for I and Se determination. Sample pre-treatment was proposed for I analysis. A digestion procedure using concentrated nitric acid, followed by extraction with ammonia was employed before the analysis [19]. Direct analysis was used for Se determination by means of TXRF, and detection limit around 0.2 mg/kg for dietary supplements was achieved [20].

### 3. CONCLUSIONS

This work, through different examples, demonstrates the suitability of TXRF for elemental chemical analysis of foodstuff and beverages. Advantages and limitations of sample preparation procedures are outlined. Recent technological improvements of TXRF allow an accurate and precise determination of major, minor and trace elements in foodstuff samples. Although, TXRF is still limited to research because of the lack of standards methods, great effort is devoted to the

development and optimization of protocols, inter-laboratory collaborations, and standards in data processing.

Studies reported in literature reveal a successful application of TXRF for food screening. This analytical technique is a suitable tool for food quality assessments thanks to its high sensitivity, reliability, lower quantification limits and fast response.

## REFERENCES

- [1] M. O. Amdur, J. Doull, C. D. Klaassen, "Casaret and Doull's Toxicology, The Basic Science of Poison's, Fourth Edit, Pergamon Press. Inc, New York, pp. 623-680, 1991
- [2] F. Guerra, A. R. Trevizam, T. Muraoka, N. C. Marcante, S. G. Canniatti-Brazaca, "Heavy metals in vegetables and potential risk for human health", *Science Agriculture*. vol. 69, 54-60, 2012
- [3] L. Borgese, A. Zacco, E. Bontempi, P. Colombi, R. Bertuzzi, E. Ferretti, "Total reflection of X-ray fluorescence (TXRF): a mature technique for environmental chemical nanoscale metrology". *Measurement Science & Technology*, vol. 20, 1-7, 2009
- [4] E. D Wannaz, H. A. Carreras, C. A. Pérez, M. L. Pignata, "Assessment of heavy metal accumulation in two species of Tillandsia in relation to atmospheric emission sources in Argentina' *Science of the Total Environment*, vol. 361, 267-278, 2006
- [5] M. Soylak, M. Tuzen, I. Narin, H. Sari, "Comparison of microwave, dry and wet digestion procedures for the determination of trace metal contents in spice samples produced in Turkey" *Journal of Food and Drug Analysis*, vol. 12, 254-258, 2004
- [6] R. A. Sánchez-Moreno, M. J. Gismera, M. T Sevilla, J. R. Procopio, "Direct and rapid determination of ultratrace heavy metals in solid plant materials by ET-AAS ultrasonic-assisted slurry sampling" *Phytochemical Analysis*, vol. 21, 340-347, 2010
- [7] M. Krachler, C. Mohl, H. Emons, W. Shotyk, "Analytical procedures for the determination of selected trace elements in peat and plant samples by inductively coupled plasma mass spectrometry" *Spectrochimica Acta Part B: Atomic Spectroscopy*, vol. 57, 1277-1289, 2002
- [8] L. Borgese, F. Bilò, R. Dalipi, E. Bontempi, L. E. Depero, "Total reflection X-ray fluorescence as a tool for food screening", *Spectrochimica Acta Part B* vol. 113, pp. 1-15, August 2015
- [9] I. De La Calle, M. Costas, N. Cabaleiro, I. Lavilla, C. Bendicho, "Fast method for multielemental analysis of plants and discrimination according to the anatomical part by total reflection X-ray fluorescence spectrometry", *Food Chemistry*, vol. 138, pp. 234-241, 2013.
- [10] R. Klockenkämper Total Reflection X - ray Fluorescence Analysis, *John Wiley & Sons*, New York, 1997
- [11] N. L. Misra, I. Varga, S. Dhara, S. K. Aggarwal, "A novel approach for chlorine determination in acidic medium by total reflection x-ray fluorescence", *X-Ray Spectrometry* vol. 38, 182-185, 2009
- [12] V. S. Hatzistavros, N. G. Kallithrakas-Kontos, "Determination of trace perchlorate concentrations by anion-selective membranes and total reflection X-ray fluorescence analysis", *Analytical Chemistry*, vol. 83, 3386-3391, 2011
- [13] K. P. Kilavi, M. D. Muchori, G. M. Gatari, A. Wagner, M. Adeleye, "Determination of Mn, Fe, Cu and Zn in Indigenous Complementary Infant Flour from Kenya by Total-Reflection X-Ray Fluorescence", *Journal of Food Nutrition Science*, vol. 2, 110-116, 2014
- [14] W. J. Langston, S. K. Spence, "Biological factors involved in metal concentrations observed in aquatic organisms", *IUPAC Series on Analytical and Physical Chemistry of Environment Systems*, vol. 3, 407-478, 1995
- [15] A. Wagner, J. Boman, "Biomonitoring of trace elements in muscle and liver tissue of freshwater fish", *Spectrochimica. Acta Part B*, vol. 58, 2215-2226, 2003
- [16] A.E.S. Vives, S. Moreira, S.M.B. Brienza, O.L.A.D. Zucchi, V.F. Nascimento Filho, "Analysis of fish samples for environmental monitoring and food safety assessment by synchrotron radiation total reflection X-ray fluorescence", *Journal of Radioanalytical and Nuclear Chemistry*, vol. 270, 231-236, 2006.
- [17] F. R. Espinoza-Quiñones, A. N. Módenes, S. M. Palácio, N. Szymanski, R. A. Welter, M. A. Rizzutto, C. E. Borba, A. D. Kroumov, "Evaluation of trace element levels in muscles, liver and gonad of fish species from São Francisco River of the Paraná Brazilian state by using SR-TXRF technique", *Applied Radiation and Isotopes*, vol. 68, 2202-2207, 2010.
- [18] T. Martinez, J. Lartigue, G. Zarazua, P. Avila-Perez, M. Navarrete, S. Tejeda, "Total reflection X-ray fluorescence analysis of trace-elements in candies marketed in Mexico", *Spectrochimica Acta Part B*, vol. 65, 499-503, 2010
- [19] I. Varga, "Iodine determination in dietary supplement products by TXRF and ICP-AES spectrometry", *Microchemical Journal*, vol. 85, 127-131, 2007
- [20] H. Stosnach, "Analytical determination of selenium in medical samples, staple food and dietary supplements by means of total reflection X-ray fluorescence spectroscopy", *Spectrochimica. Acta Part B*, vol. 65, 859-863, 2010.