# NUTRITIONAL MINERAL IN FOODS BY TXRF: AN EASY, FAST AND RELIABLE APPROACH FOR FOOD CHARACTERIZATION

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***Abstract*** – The mineral nutritional values of foods is an important aspect that should be considered (especially the intake for iron, calcium, potassium, manganese, copper, chromium and zinc) to obtain a correct balanced diet, that is essential to maintaining good health.

In the literature there are many information on mineral content of different foods, but due to the high analytical costs of traditional methods (AAS, ICP-MS) in the real world, only few data are routinely produced.

The use of an alternative cheap analytical technique as TXRF can help to overcome this knowledge gaps. [1-8]

The TXRF quantification were improved using triplicates analysis and two internal standard, the first (Gallium) for quantification and the second one (Vanadium) for verify the accuracy and repeatability of method.

The results show that TXRF technique is reliable and could be successfully used for determination of main mineral contents in food, and also for trace elements.

Finally, a complete mineral characterization can be used to define the authenticity and can help for traceability of food products

***Keywords***: TXRF, food, nutritional mineral, slurry pretreatment

## 1. INTRODUCTION

X-ray fluorescence (XRF) techniques and especially Total Reflection X-Ray Fluorescence (TXRF), offer a real alternative for a rapid and efficient food analysis. Using XRF it is possible to measure almost all the elements from the periodic table formally from Aluminium to Uranium; the use of total reflection configuration the detection limits are improved by reducing the matrix effect. Recently these technology are commercially available in compact bench-top instrumentation.

Instead to AAS technique that allows mono-elemental analysis, TXRF like ICP can provide a simultaneous multi-elemental analysis.

One other main advantages are that TXRF calibration procedures are simpler than ICP, and that digestion of organic materials is not a mandatory sample pretreatment prior analysis.

Slurry sampling is simpler, safer and faster than acid digestion that use concentrated acids at high temperature and pressure.

The aim of our work is to verify the TXRF performance for the assessment of nutritional elements in many different food types as: row materials, processed foods and novel foods matrices using a multi-elemental TXRF equipment combined with simple slurry pretreatment.

## 2. MATERIAL AND METHODS

### 2.1. Reagents and standards.

All chemicals were of analytical-reagent grade. Ultrapure water was obtained from MilliQ system (Millipore, Bedford, MA, USA). Triton X-100 (Merck, Darmstadt, Germany) and Germanium standard solution for ICP 1000 mg/L), and Vanadium standard solution for ICP 1000 mg/L (Ultra Scientific North. Kingstown, RI, USA) were used.

Silicon solution in isopropanol (Serva, Heidelberg, Germany) was used to make the carrier surface hydrophobic and to reduce the sample drop spreading on its surface.

The following certified reference materials were used for method validation:

- SRM1515 Apple Leaves,
- SRM1570 Spinach Leaves,
- SRM1547 Peach Leaves,
- DORM3 Fish protein ,
- DORM4 Fish protein ,
- SRM1568 Rice Flour,
- SRM2976 Mussel Tissue

- BCR185r Bovine liver.

2.2. Instrument

Slurry was prepared using PrioGENIZER homogenization device (Prionics AG, Schlieren, Switzerland).

Analysis was carried out with a Bruker S2 Picofox TXRF spectrometer (Bruker GmbH, Berlin, Germany). The TXRF spectrometer is equipped with a Mo tube (1000µA, 50 kV, 50 W), a multilayer monochromator and a high sensitivity silicon-drift detector.

2.3. Analytical procedure

Concentrations of 14 elements (P, S, Cl, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Br, Rb, and Sr) were determined in food samples by Total Reflection XRay Fluorescence (TXRF).

A sample preparation procedure based on slurry was developed as an alternative to the classical sample digestion method.

To verify the performance of method and sample deposition, that represent the critical point of TXRF methodology, it has been necessary to introduce not only the usual Gallium internal standard for quantisation but another unusual metal as Vanadium as internal check.

Ga has been shown to provide good results for all foodstuff tested. To avoid signal was overlapped by the Kβ peak of Ga its concentration was reduced till signal reproducibility produce a good quantification.

2.4. Sample preparation

A 50 mg portion of sample was accurately weighed on an analytical balance and placed in a 5 mL and the PrioCLIP™ tube.

Then, 4 mL of a surfactant solution was added (0.5 % w/v Triton X-100 solution and 40 µl of Standard mix solution 2,5 mg/L of Ga and 10 mg/L of V as internal standards.

For each sample three replicates were performed. The sample mixture was subjected a two cycle of revolution speed profile reaching 18000 rpm for 120s.

An aliquot of 10 µL of the suspension was withdrawn immediately after stopping mixing. The aliquot was placed in the center of a disposable Plexiglas reflector (30mm diameter and 3mm thickness) previously siliconized, and dried gently on a hot plate until complete evaporation.

Dried sample reflector's were introduced in 25 sample holder into the TXRF instrument.

A lifetime of 1000 s was used.

For spectra evaluation and quantification Spectra® 7.5. Bruker Nano GhbH software was employed. Quantification was performed using a deconvolution based on a Bayesian inference (Profile Bayes-normal fit).

## 3. RESULTS AND DISCUSSION

Many parameters were studied in order to optimize the slurry procedure, especially Triton X-100 concentration, total volume and sample mass employed.

Sample masses of 25, 50 and 100 mg were tested. For a sample mass of 100 mg, a non-homogeneous distribution of particles was visually observed, and matrices effect were detectable.

Sample mass of 50 mg of wet material has be used for quantitative purpose. In cases of dry material low mass was used (25mg).

Method validation was performed by analysing eight Certified Reference Materials. The criteria used to verify the method suitability was an average recovery in the range 80-120%. All the 14 elements fulfilled this criteria.

Table 1. recovery Certified Reference.

| | | SRM 1515 | SRM 1570 | SRM 1547 | DORM 3 | DORM 4 | SRM 1568 | SRM 2976 | BCR185r |
|---|---|---|---|---|---|---|---|---|---|
| P | 80 | 103 | 77 | 91 | | | 71 | | |
| S | 102 | 132 | | 87 | | | 86 | | |
| Cl | 95 | 106 | | 83 | | | | | |
| K | 101 | 125 | 92 | 94 | | | 94 | | |
| Ca | 96 | 71 | 87 | 83 | | | 142 | | |
| Mn | 91 | 98 | 92 | 84 | | | 90 | | 92 |
| Fe | 88 | 79 | | 95 | 74 | 81 | | 110 | |
| Ni | 118 | 151 | 115 | | 117 | 95 | | 110 | |
| Cu | 101 | 104 | 106 | 109 | 101 | 89 | 135 | 87 | 81 |
| Zn | 101 | 119 | 96 | 99 | 104 | 81 | 109 | 106 | 91 |
| Br | 97 | 107 | | 87 | | | | | |
| Rb | 83 | 95 | | 80 | | | 74 | | |
| Sr | 84 | | 84 | 84 | | | | | |

The high variability visible in table 1 could be partially explained linking to the concentration of a single element present in different matrices (data not show).

The final routine quality check for each sample are: Repeatability of the three replicates within ±15 % and recovery of Vanadium within 85-115 %

A first estimated uncertainty budget is around 20%, and the main identified sources of uncertainty are:

- the representativeness of laboratory sub sampling, because we work on a very small amount of sample and replicates are suggested.
- the quality of sample slurry homogenization, depending on equipment used.
- the deposition on carrier surface
- the measurement repeatability

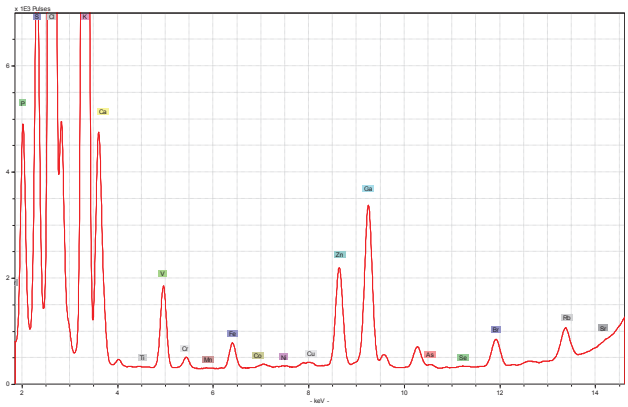

Fig. 1. Salami TXRF spectrum

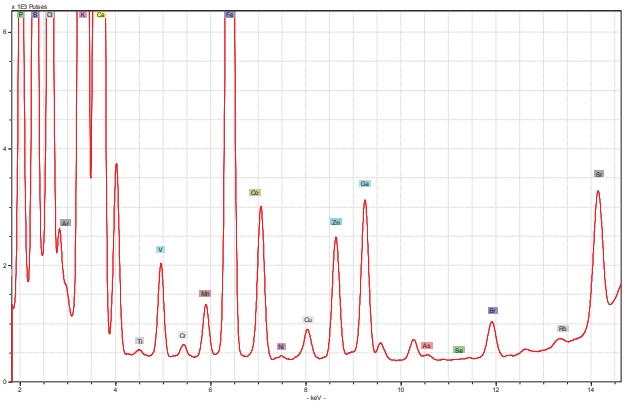

Fig. 2. Spirulina algae TXRF spectrum

## 4. CONCLUSIONS

The Slurry sampling procedure and TXRF technique allows a successful measurement of 14 elements in different food matrices, i.e., P, S, Cl, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Br, Rb, and Sr.
The method were tested with good results on a large variety of food products such as raw meat, salami, sausages, fish, mussels, squid, milk and cheese, wine, carrots, berries, ice-cream, honey, cocoa, coffee, olive oil, biscuit, cakes, spirulina algae and cricket flour.

In figures 1 and 2 were show example of salami and spirulina sample prepared as described.

It is possible underline than internal standards Ga and V fall in a relatively clear energy ranges of spectra.

In sausages and salami spectra beyond the nutritional composition it is possible to check easily the presence of additives as polyphosphates (P), verify the content of salt (Cl), identify the presence of Mechanically Separated Meat (Ca) and detect some accidental metal contaminations (Cr, Co, Ni)

Similar evaluation could done for milk product hard and soft cheese for additives (polyphosphates), salt content or identification of milk species.

We tested many different fish species both Freshwater and Saltwater fish (eg. Salmon, Mullet, Mussels, Swordfish, Tuna, Squid etc), and each fish species or group of fish present its characteristic mineral profile. In some case beyond the mineral nutritional value it is possible to verify the quality and the food safety of fish product, for example the Squid spectra is very plain and the arsenic signal is well defined, it is possible to check easily its concentration around the maximum residue limit.

Also vegetables like chicory, carrot, lentils, green and roasted coffee can be processed for TXRF analysis with the same procedure.

As in previous food categories each vegetable present a specific profiles in terms of element concentrations and ratio between them.

The small amount of sample needed, allow to analyzing high costly product as saffron or other spices

A different extraction treatment of virgin olive oil allow to determine its water soluble mineral fraction.

The spectra of PDO/PGI o artisanal virgin olive oil present a very clean spectra, compared to industrial product where the signals of some mineral (eg. Zn, Ca and Fe) were more noticeable.

Using TXRF it is possible to explore without problems the new frontiers of food product like cricket flour or spirulina algae product.

It is possible to determine and to compare the nutritional strength of these novel food.

Furthermore the determination of not usually checked elements such as Br, Sr and Rb seem to be very related and useful for traceability or geographical characterization of food products.

All these elements were determined simultaneously without digesting the sample and with a minimum use of reagents and low waste.

Analysis time for each sample (three replicates per sample) was less than 45 min, including weighing, slurry preparation, measurement and data interpretation.

Using a disposable Plexiglas reflector the quality of data seem to be not affected and it simplify the procedure, prevent cross contamination and produce a general time saving.

The estimated uncertainty of overall analytical protocol seem to be good enough for screening purpose and for food characterization.

The next step of research will be focused on optimizing and improve the slurry homogenization process and to define a simple and more reliable sample deposition procedure (manual and/or automatized)

The proposed procedure is applicable to an huge range of food product, is fast, cheap and represent a new, powerful tool in food routine controls for consumer protection.

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