TXRF ANALYSIS OF FOOD AND BEVERAGES: THE IZSLER EXPERIENCE

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Abstract –
TXRF (Total X-Ray Reflection Fuorescence) is a simple and fast method to analyse elements. Different types of food and beverages were analysed with TXRF to verify the concentration of some elements and to point out possibly differences of composition. Encouraging results were obtained in combination to multivariate analysis of data too.

Keywords: TXRF, food, statistical analysis

1. INTRODUCTION

Nowadays there is the need to analyse food without manipulations that can alter its integrity, along with simple and fast methods. TXRF(Total X-Ray Reflection Fuorescence) can fit this features.

TXRF, with an X-ray incident beam below the critical angle to the sample, minimize the high background of traditional X-ray fluorescence (xrf) through total reflection of the primary radiation and offers surface sensitive, ultra trace element analysis. Since the wavelength and energy of the fluorescence radiation are specific for each element, TXRF analysis is possible because the concentration of each element can be calculated using the intensity of fluorescence radiation.

The aim of this work is to demonstrate the feasibility of TXRF analysis of different type of food and beverages highlighting problems and facilities of this technique.

2. MATERIALS AND METHODS

2.1. Equipment

MM 400 mixer ball mill (Retsch)
PrioGENIZER homogenization device (Prionics)
TXRF S2 Picofox spectrometer(Bruker Nano Gmbh)
Mo-X-ray tube, multilayer monochromator and silicon drift detector.

2.3. Reagents

Silicon Solution in isopropanol (SERVA)
Triton X
Polivinilalcool

2.4. Sample preparation

Each sample was analysed in triplicate

The sample carrier (quartz and plexyglass type) was previously cleaned and controlled to avoid interferences from contamination and siliconized with 10 µL of Silicon solution in isopropanol.

Wine: 990 µL of wine was mixed with 10 µL of Ga standard solution (100 mg/L) and stored in 1.5 mL Eppendorf tubes. The final concentration of Ga in each specimen was 1 mg/L; 10 µL were pipetted at the centre of a siliconised sample carrier, and dried at 50 °C.

Coffee: 990 µL of coffee solution was mixed with 10 µL of Ga standard solution (100 mg/L) and stored in 1.5 mL Eppendorf tubes. The final concentration of Ga in each specimen was 1 mg/L; 10 µL were pipetted at the centre of a siliconised sample carrier, and dried at 50 °C.

Balsamic vinegar: 100 µL of vinegar was mixed with 10 µL of Ga standard solution (100 mg/L) and stored in 1.5 mL Eppendorf tubes. The final concentration of Ga in each specimen was 1 mg/L; 10 µL were pipetted on a siliconised sample carrier, and dried at 50 °C.

Honey: 500-1000 mg are solubilized 1:5 with triton-X 1% in water and polivinilalcool (pva) 0,2%, 10 µl of

internal standard (100 ppm Ga) were added. Finally 10 µL of the solution were pipetted at the centre of a siliconised sample carrier

Cheese: the sample was previously homogenated with a grinder, 200 mg were suspended in water/ triton-X 1% and polivinilalcool (pva) 0,2%, 10 µl of internal standard (100 ppm Ga) were added. The suspension was homogenated 10 min in a PrioGENIZER. Finally 10 ml was pipetted on the sample carrier

Meat and processed meat: the sample was previously homogenated with a grinder, 200 mg were suspended in 4 mL water plus 0,5 mL triton-X 1% and 0,5 mL polivinilalcool (pva) 0,2%, 10 µl of internal standard (100 ppm Ga) were added. The suspension was homogenated 10 min in a PrioGENIZER. Finally 10 µl was pipetted on the sample carrier

Oil: 5 g of oil was extracted with 0,5 mL of MQ water, after vortex-shaking and centrifugation 200 µL aqueous phase was added with 20 µL of internal standard (Ga solution at 10 mg/L) and mixed; 10 µL was deposited on a plexiglas disk (sample carrier) and let dry before the analysis.

2.5. TXRF analysis

Experimental conditions:
Excitation setting 50 kV and 600 mA
Gallium solution was used as Internal Standard
Sample carriers: acrylic glass disks were used
SPECTRA software (Bruker) was used for the deconvolution of the spectra and quantification.

Analysed elements: Mg, P, S, K, Ca, Ba, Cl, Br, Mn, Fe, Ni, Cr, Cu, Al, Zn, As, Ti, Sn, Rb, Sr,, I and Pb. Among them Mg, Al, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, As, Br, Sr were calibrated and recorded using K series; Sn, I, Ba and Pb were calibrated and recorded using L series

Quantification: the instrument was calibrated with certified single element solution, calibration was corrected with certified materials. The element concentrations was calculated using Gallium as internal standard

Sensitivity: the TXRF sensitivity is correlated to the atomic number so the heaviest elements have more response to X-ray and are more detectable.

With Mo-K excitation spectrometer the elements with lower atomic number (≤ 39 Yttrium) are detected with their K-lines, meanwhile from Zirconium (atomic number 40) L-lines are used. The element sensitivity and their detection limits vary over a broad range of values. A sensitivity factor S_i is calculated for each element with a measurement of Gallium as internal standards and all other elements at different concentration using the following equation

$$Si = \frac{N_i \; x \; C_{Ga}}{N_{Ga} \; x \; C_i}$$

where N_i are the net counts of the i element peak, N_{Ga} the net counts of the Ga peak, C_i the concentration of the i element and C_{Ga} the concentration of Ga.

Quantification: The quantification of the elements was determined with the equation

$$Ci = \frac{N_i \; x \; C_{Ga}}{N_{Ga} \; x \; C_i}$$

where C_i is the concentration of i element, , S_{Ga} is the relative sensitivity of Ga, N_i are the net counts of the i element peak, C_{Ga} is the Ga concentration, N_{Ga} the net counts of the Ga peak, S_i is the relative sensitivity of i element

2.6. Statistical analysis

Unscrambler 10.2 software (CAMO software) was used

3. RESULTS AND DISCUSSION

Sample preparation

Due to the limited amount of sample that is analysed (from theoretical 100 µg for liquid sample to 0.4 µg for solid samples), the representativeness was achieved with analysis in triplicate. Different approaches were used for the preparation of different type of food and beverages: for the water soluble liquid food a simple water dilution with the aid of surfactant is sufficient for the analysis (wine, balsamic vinegar, coffee), for solid samples (cheese,

meat and processed meat) after the homogenisation the samples is suspended with a specific homogenizer at high velocity and the suspension is pipetted on the sample carrier; the extraction with water for the water soluble components was used for oils too.

The use of a ball mill (e.g. Retsch MM 400) for the solid sample preparation is a possible implementation of the analysis in terms of repeatability: with this type of homogenization it is possible to reach the particle size that allows a good suspension. The better homogeneity of the samples had as a counterpart a possible cross-contamination from other samples. To prevent this possibility it is necessary to accurately wash the milling chamber and balls. About the release of impurity from the mill component itself(zirconium oxide) no specific study were conducted but it seems not a really problem.

Wine: nine Italian wines were analysed to verify the concentration of some elements. Quantitative analysis of Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr and Pb was performed.
Since no certified reference materials (CRMs) was available for trace elements determination in wine, for the accuracy determination a 12% alcoholic solution was added to the ICP multi-element standard solution (IV, 23 elements in diluted nitric acid) to prepare two reference solutions with concentrations of about 10 and 0.1 mg/L. The obtained results were from 69±0,6 % (Cd) to 100±0,5 % (Fe) of the expected value.
Traces of Fe, Cu, Zn and Pb are present in all the analysed samples.

Honey: 134 monofloreal honey were analyzed to verify the possibility to distinguish the different botanical species and to detect possible adulteration. P, Cl, K, Ca, Fe, Zn were detected in all the analyzed samples, S and Cu were missing in some samples. P, Cl, K and Ca were the most abundant elements. Statistical analysis of the data is ongoing.

Balsamic vinegar: Protected Geographical Indication (PGI) balsamic vinegar is made using grape must, vinegar, caramel could be added and the seasoning is shorter, at least two months. The analysis of the element composition could differentiate the industrial made PGI vinegar from the artisanal type one. Na, P, S, Cl, K, Ca, Mn, Fe, Zn was detected in concentration varying from hundreds of mg/kg for Na, K, P and Cl to less than one mg/kg for Mn and Zn. The number of samples must be implemented to have a more precise profile of this dressing.

Cheese: aim of this work was to differentiate two very similar cheese, grana padano and Trentingrana cheese, with the analysis of its elements. 25 Trentingrana cheese, 35 Grana Padano cheese and 2 mixtures were analized.

The spectra of a Grana Padano cheese is reported in fig 1

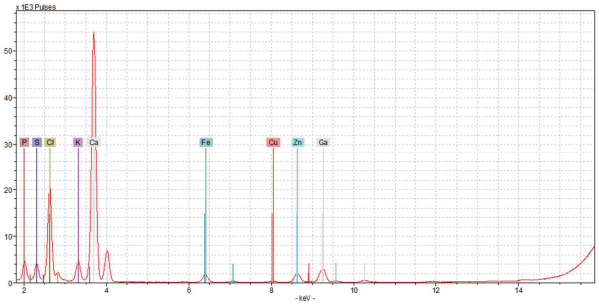


Fig1: grana Padano cheese spectra

The autoscaled data were treated with PCA.
To the calculated score Linear Discriminant Analysis (LDA) was applied. The classification was good with all the Trentingrana cheese. Grana Padano cheese was well classified with two grana Padano confused with Trentingrana only. The mixture were overlapped with Grana Padano too. The Classification matrix is in table 1

Table 1 Classification matrix of cheese samples

	NER%	Mixture	Padano	Trentingrana
Mixture	50	1	1	0
Grana Padano cheese	94.28	0	33	2
Trentingrana cheese	100	0	0	25
Total	95.16	1	34	27

It was not possible to highlight differences in seasoning production and producers.

Meat. aim of this part of the work was to detect mechanically separated meat throw the concentration of P and Ca that could derive from the bone “grasped” in mechanical processes. The analysis data show a considerable difference of Ca content between meat and mechanically processed meat. Statistical treatment separates in a better way the two type of food as shown in fig.2

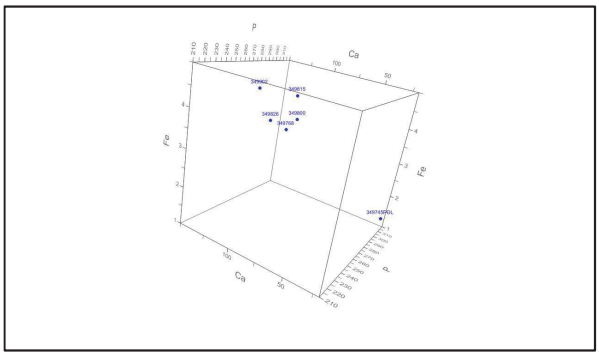


Fig2. tridimensional plot of PCA for meat

Another aim was to separate the different mechanical treatment of the meat (high and low pressure)

On the base of the UE regulations, the mechanically processed meat or mechanically recovered meat can be obtained with the application of high and low pressure. The high-pressure mechanically separated meat is paste-like and can be used in products such as hotdogs; the low-pressure mechanically separated meat is similar in appearance to minced meat. This two different products are distinguishable in relation to bone structure alteration and calcium content. High pressure production processes increase the risk of microbial growth. In fact these processes result in greater muscle fiber degradation and an associated release of nutrients which provide a favorable substrate for bacterial growth. For this reasons it is very important to discriminate high and low pressure products in a rapid and affective way.

Oils: the olive oils were from retail and from local PDO producers.

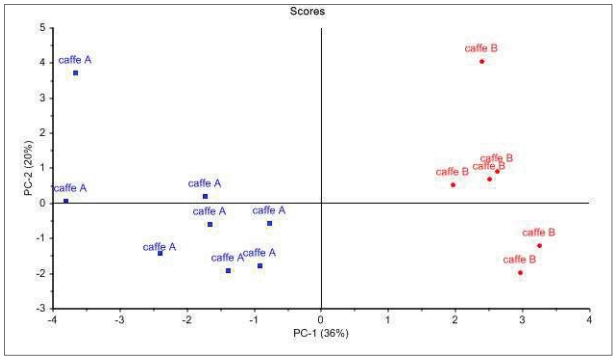
93 extra virgin olive oils

17 olive oil (a mixture of refined oils and extra virgin oils) were analysed.

Given that only a minor part of the olive production is fit for extra virgin oil, the olive oil treatment (bleaching, neutralizing, degumming, deodorizing) is an important sector of oil industry and a risk for frauds and counterfeits. Several elements are implicated in the oil industrial treatment with e.g. bleaching could remove metals like iron and copper, the removal of gums is aided with the use of phosphoric acid (in order to remove phosphatides). Some elements detected in the oils could be related to this processes and some others like Mn, Fe, Zn, Br, Sr and Ba could be related to EVOO geographical origin.

In the analyzed samples, between olive oils and (declared) extra virgin oil there are differences for P, K, Ca, Fe, elements related to industrial processing. Among elements related to the geographical origin, Mn, Ba, Zn and Cu show the most pronounced differences, meanwhile Mg and Al were detected in few samples only.

Coffee: 14 samples of extract of two different type of coffee, Arabica and robusta, were analysed. With a simple PCA it was possible to differentiate the two quality of coffee as depicted in figure 3.



4. CONCLUSIONS

TXRF has demonstrated to be a friendly and powerful tool of analysis for different types of food and beverages.

For quantitative analysis of elements there is the need of a good calibration with certified materials and the use of the right internal standard.

For qualitative analysis the correct use of statistical analysis is mandatory along with better sample preparation that means better reproducibility.

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Fig. 3 score plot of coffee