A SMART SAMPLE PREPARATION METHOD FOR CHOCOLATE ANALYSIS

Fabjola Bilo [1], Laura Borgese [1, 2], Rogerta Dalipi [1], Annalisa Zacco [1, 2], Elza Bontempi [1, 2], Laura E. Depero [1, 2]

[1] Chemistry for Technologies Laboratory, Department of Mechanical and Industrial Engineering, University of Brescia, Brescia, Italy,
email: f.bilo@unibs.it
[2] SMART Solutions s.r.l, Brescia, Italy

Abstract – Fast, simple and reliable methods are necessary for quality control of food sample that will guarantee the food safety and human health. Total reflection X-Ray Fluorescence (TXRF) spectroscopy is suitable for elemental analysis of food, since it provides simultaneous multi-elemental identification in a wide dynamic range of concentration. Moreover, TXRF is successfully applied for sample screening. This study proposes a new approach of sample preparation procedure developed by Smart Store for elemental chemical analysis of foods by means of TXRF. The method consists in sandwiching the chocolate sample into two polymeric foils, free from heavy metals content, allowing to protect it from external contamination, to avoid any material loss and to store it for further investigations. After preparation, the samples can be directly analysed. Furthermore, in this work the total solubilisation of the samples by microwave acid digestion was performed. Quantitative analysis by means of internal standard addition was performed for all samples. Good agreement results was achieved for both sample preparation procedures.

Keywords: heavy metals, direct analysis, TXRF, chocolate, screening

1. INTRODUCTION

Human health is strongly dependent on the quality of daily consumed food. Since food is one of the main sources of essential elements intake for humans, it should guarantee the absence of contaminants and the presence of nutrients. However, it may be contaminated by potentially toxic metals during the production or storage process.

Chocolate is one of the favourite food consumed by all consumers age. It dates back to about 1500 years ago. Firstly it was used by Mayan and Aztec Civilization as a drink. Nowadays, in the market are available many types of chocolates such as dark, semisweet, bittersweet, baking and white. The latter one is technically no real chocolate since it does not contain chocolate liquor. Usually, a list of ingredients is declared on the wrappers. Among the main constituents are: sugar, cocoa solids, hydrogenated vegetable oil (HVO), vegetable fats, salts, buffering agents, permitted stabilizer, sodium bicarbonate, cocoa butter, wheat flour etc. Unfortunately, some of them such as cocoa solids, cocoa butter, buffering agents may be the source of heavy metals (Cd, Pb, Ni) contamination [1]. Therefore, the determination of the elemental composition of chocolate is mandatory to make recommendations for its consumption, in particular with respect to children, pregnant women and people with allergies to specific element. In this frame, recent trends in food safety issues have generated concerns over the presence and levels of heavy metals in cocoa. International legislative bodies have already introduced new regulations for the health protection of their consumers [2].

Elemental chemical analysis of foodstuff samples is a complicated process, which highly dependent on the sample matrix. Most of analytical methods for metal analyses in cocoa require the sample solubilisation, and elemental determination is performed by electrochemical [3], atomic absorption spectroscopy (AAS) [4], and inductively coupled plasma mass spectrometry (ICP-MS) [5]. Nowadays, easy, sensitive and reliable analytical techniques are required as a result of the increasing demand on multi-elemental information and food screening. In this sense, Total reflection X-Ray Fluorescence (TXRF) spectroscopy may be used as a suitable technique that fulfils the above mentioned requirements. Recent trends of TXRF application for foodstuff analysis have been reviewed [6].

The key advantages of TXRF compared to other elemental analytical techniques are: possibility of direct analysis, simultaneous multi-element trace analysis including halogenides; analysis of very small sample amounts (ng or mg); simple quantification using an internal standard; suitable for various sample types and applications; theoretically no matrix or memory effects; low operating costs; fast, and easy sample preparation procedure, short time analysis, high sensitivity and low detection limits in the order of ppb, depending on the sample (elements) and the matrix [6, 7, 8].

The aim of our work is to demonstrate the suitability of this new sample preparation strategy for direct elemental analysis of chocolate by using TXRF spectroscopy, reducing the difficulties we are facing during the conventional methods, facilitating sample storage, cost effectiveness.

2. MATERIALS AND METHODS

2.1. Chocolate Samples

Seven dark chocolate and one milk chocolate bars were employed to study the suitability of the proposed sample preparation procedure. The sample list is reported in Table 1.

Table 1. Brand of chocolate samples and their origin country.

Number	Chocolate Brand	Type	Cacao %	Produced Country
1	Le bon	Dark	50	Italy
2	Chocolat	Dark	52	UE
3	Le petit chocolateir	Dark	45	Switzerland
4	Wawel	Dark	90	Poland
5	Deluxe	Dark	60	Belgium
6	Dolciando	Dark	50	Italy
7	La suissa	Dark	52	Italy
8	AOHNAI	Milk	25	Greece

2.2. Microwave Acid Digestion

All chocolate samples were digested according to EPA 300.2 method. Approximately 0.4 g of chocolate samples were put in Teflon vessels, added with 6 mL of concentrated nitric acid (65% - Sigma Aldrich) and 2 mL of H_2O_2 (37% - Sigma Aldrich). Samples were digested using CEM SP-D microwave system. Each sample was individually processed. A five steps procedure was automatically performed to have complete digestion: 3 min at 160 ^{0}C, 5 min at 180 ^{0}C, 3min at 200 ^{0}C, 5 min at 205 ^{0}C, and 10 min at 210 ^{0}C. After cooling, the volume of each sample was adjusted to 25 mL adding MQ water.

Quantitative analysis was performed using Ga as internal standard element (IS), in final concentration 1 mg/L. Therefore, an aliquot of 1 mL digested sample was spiked with 10 µL of 100 mg/L Gallium in acid nitric (Ga-ICP Standard Solution, Fluka, Sigma Aldrich) solution. 10 µl of Silicone solution in isopropanol (Serva Electrophoresis, Heidelberg, Germany) was deposited on cleaned quartz glass reflectors with aim to obtain a hydrophobic surface. A drop of 10 µl of the prepared solution was deposited in the centre of the prepared reflector and dried on a hot plate at 50 °C. Three trials were prepared and measured for each sample.

2.3. Smart Store procedure

About 6 g of chocolate was mixed with 3 ml of 100 mg/L Ga solution in a beaker and dissolved in bain-marie conditions. Then, around 0.2 g of total dissolved sample was placed on polymeric foil and dried on environmental temperature. Samples prepared as described above, were sandwiched between two foils of polypropylene [9]. In the meanwhile Smart Store labels the plasticized sample with a QR code and classifies it to the respective category. The obtained thin sample was placed onto a quartz sample carrier with a diameter of 30 mm and inserted into the instrument for measurement (Fig.1).

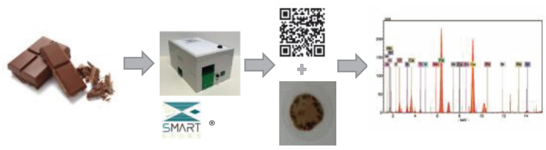

Fig. 1. Sample preparation of chocolate according to Smart Store method.

2.4. Instrumentation.

TXRF measurements were carried out by a Bruker S2 Picofox spectrometer (Bruker AXS Microanalysis GmbH, Berlin, Germany), equipped with a Mo tube operating at 750 µA and 50 kV, multilayer monochromator, silicon drift detector (SDD) and energy resolution was 165 eV at 5.9 keV. Samples were irradiated for 600 s live time.

3. RESULTS AND DISCUSSION

The raw spectra of TXRF measurements performed for digested and plasticized chocolate sample are shown in Figure 2. Peak identification and automatic qualitative analysis was performed by using the qualitative scan mode linked to the equipment. This step gives a multi-elemental information and is useful for screening of the studies samples. The following elements were identified in all the analysed samples: K, Ca, Ti, Mn, Fe, Ni, Cu, Zn, Br, Rb, and Sr. While presence of P, S, Ti, Cr and Ba was observed only in digested samples, probably due to a lower detection limit. The intense X-ray peak at around 1.7 keV is due to the Si-Kα from quartz sample carrier, the peak of Ar-Kα from air and of Mo-Kα from X-ray tube are also presented. Spectra comparison for chocolate (CH1) sample prepared according two sample preparation methods highlights the repeatability the proposed procedure. The chemical information in this approach is similar to that obtained by digested sample analysis.

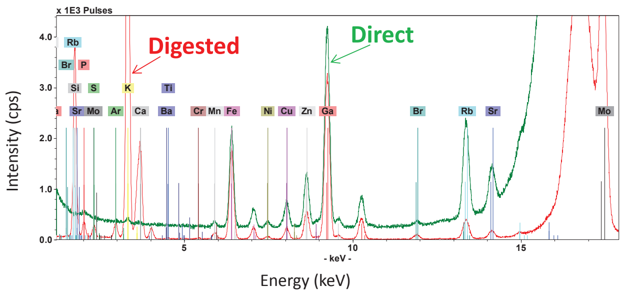

Fig. 2. TXRF spectra of digested (red) and plasticized (green) chocolate (CH1) sample.

Comparison of two reported spectra points out the main drawback of the plasticized samples: the higher background, which is probably due to a lower homogeneity of the sample.

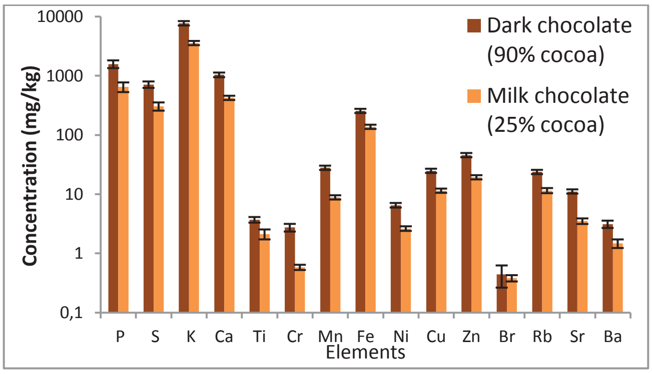

Fig. 3. Elemental concentration in dark and milk chocolate digested sample measured by TXRF

Figure 3 shows the elemental concentration (mg/Kg) in two different chocolate types. Trend of growing of the concentration by the growth of the content of cocoa in samples of different types was found. Slightly lower metals content was observed in milk chocolate. Results of major and minor elements content in 8 digested chocolate samples are reported in Figure 4. K was found to be the most abundant element in all chocolate matrixes, followed by P, Ca, S and Fe. While, in the group of trace elements, Zn shows the highest concentration with respect to others. Presence of potentially toxic elements like Cr and Ni, was also detected in the analysed samples. The lowest concentration was observed on milk chocolate, probably due to the lower content of cocoa butter in this sample [1]. The contamination sources for these metals are mainly the raw materials used, manufacturing processes, as well as leaching of these metals from the vessels in which they are stored. Processing of chocolates is done in steel containers from which nickel contamination may occur. However, concentration of these elements is comparable to those reported in other studies [1, 10, 11].
For this reason, the daily intake of chocolate products should be reduced, in order to keep the heavy metals concentration within the permitted levels. Nowadays, there are no well-defined limits for concentration of toxic elements in chocolates in most of the countries.

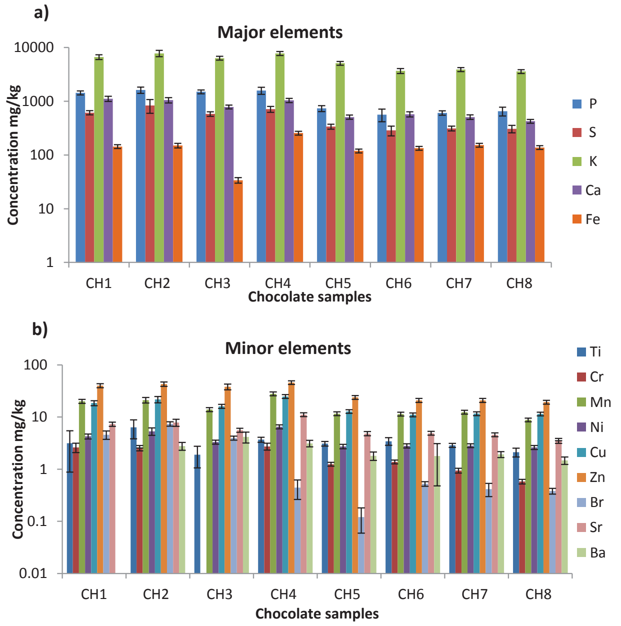

Fig. 4. Concentration of major (a) and minor (b) elements in digested chocolate sample measured by TXRF

The metal concentrations in digested samples were determined even by Synchrotron Radiation Total Reflection X-Ray Fluorescence (SR-TXRF). Samples were excited with energy 14 keV operating under vacuum and total reflection conditions. Comparison of elemental concentration in chocolate obtained by SR-TXRF and TXRF is presented in Figure 5.

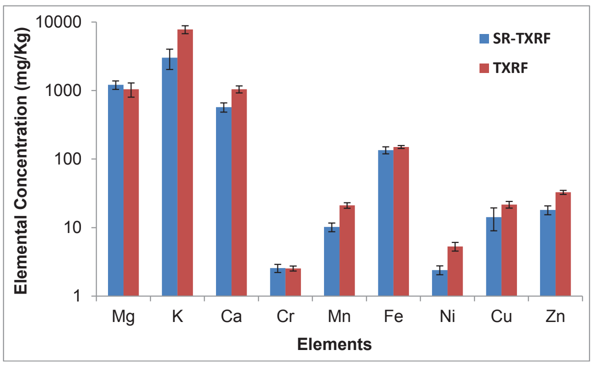

Fig. 5. Elemental concentration in digested chocolate obtained by SR-TXRF and TXRF.

Good agreement results between two techniques were achieved. Determination of Rb and Sr was not possible by SR-TXRF due to the energy excitation. While the plasticized sample was not irradiated since that working conditions were not appropriate.

As far as we know, there is no paper in the literature dealing with the direct analysis of chocolate samples.

Indeed, the novelty of our suggested approach for chocolate sample preparation is that samples may be analysed directly by different XRF instruments configuration and did not require chemical pre-treatment to leach elements from the solid sample as usually are required in other spectroscopic techniques such as FAAS. In Figure 6 are reported results of TXRF analysis for both discussed sample preparation methods. A semi-quantitative analysis is performed for plasticized chocolate samples. Our data reveal that light elements like K and Ca are underestimated compared to digested samples, probably due to a higher absorption effect than heavier metals. In plasticized sample, the thickness increases causing an increase of the radiation intensity, and this is a source of absorption or enhancement effects.

We are still working to evaluate the relative sensitivity of elements and to determine the matrix effects, which will led to improve the limitations of the suggested method.

Despite of this, the obtained results suggest that this method can be proposed as a suitable tool for a reliable sample screening at very low concentrations.

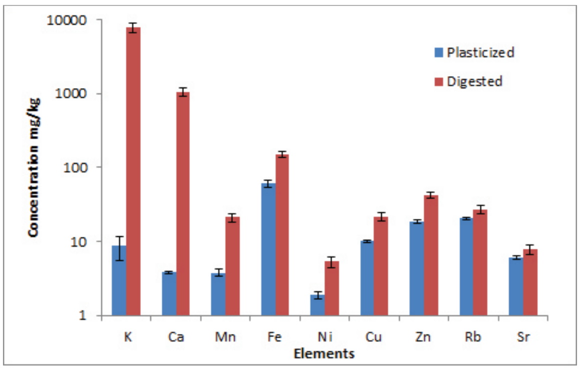

Fig. 6. Elements concentration in chocolate sample: comparison between new approach and conventional method.

4. CONCLUSIONS

In this work, a reliable sample preparation method was developed for screening analysis of chocolate samples. Moreover, after the analysis, the sample can be archived for subsequent re-analysis.

Results of our study suggest the suitability of the proposed procedure as a fast and sensitive method for heavy metals determination in this kind of foodstuff samples. Evaluation of relative sensitivity and matrix effects are still ongoing. In this way, this approach may offer new possibilities even in other food samples.

Our data show that the elemental concentration of all the analysed chocolate samples is similar independently from the origin country.

REFERENCES

[1] S. Dahiya, R. Karpe, A. G. Hegde, R. M. Sharma, "Lead, cadmium and nickel in chocolates and candies from suburban areas of Mumbai, India" *Journal of Food Composition and Analysis*, vol. 18 pp. 517–522, 2005

[2] European Food Safety Authority [EFSA], Metal Contaminants in Food. Retrieved, 16 April, 2011 from: http://www.efsa.europa.eu/en/topics/topic/metals.htm

[3] I. Karadjova, S. Girousi, E. Iliadou, I. Stratis, :Determination of Cd, Co, Cr, Cu, Fe, Ni and Pb in Milk, Cheese and Chocolate" *Microchimica Acta*., vol. 134, nº 3, pp. 185-191, 2000

[4] K. Srogi "Assessment of Selected Heavy Metal Contents in Medicinal Plants, Tea Leaves and Chocolate using Atomic Absorption Spectrometry" *Acta Toxicologica*, vol. 14, pp. 117-128, 2006

[5] S. Mounicou, J. Szpunar, R. Lobinski, D. Andrey, C. J. Blake, "Bioavailability of Cadmium and Lead in Cocoa: Comparison of Extraction Procedures Prior to Size-Exclusion Fast-Flow Liquid chromatography with Inductively Coupled Plasma Mass Spectrometric Detection (SEC-ICP-MS)", *Journal of Analytical Atomic Spectrometry*, vol. 17, pp. 880-886, 2002

[6] L. Borgese, F. Bilo, 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

[7] 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.

[8] R. Klockenkämper Total Reflection X – ray Flourescence Analysis, *John Widely & Sons*, New York, 1997

[9] L. Borgese, A. Zacco, S. Pal. E. Bontempi, R. Lucchini, N. Zimmerman, L. E. Depero, " A new non-destructive method for chemical analysis of particulate matter filters: The case of manganese air pollution in Vallecamonica (Italy)", *Talanta*, vol. 84, pp. 192–198, January 2011

[10] R. LeviYanus, H. Sela, E. J. C. Borojovich, Y. Zakon, M. Saphier, A. Nikolski, E. Gutflais, A. Lorber, Z. Karpas, "Trace elements in cocoa solids and chocolate:An ICP-MS study", Talanta, vol. 119, pp. 1-4, 2014

[11] N. Alagić, J. Huremović, "Determination of metal contents in various chocolate samples", *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina*, vol.45, pp. 39-42, 2015.