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Analytical note

Trace element determination in soy sauce: A novel total reflection X-ray fluorescence procedure and comparison with inductively coupled plasma–mass spectrometry☆

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ABSTRACT

Soy sauce is a widespread food commodity very common in East and Southeast Asia. It features a complex matrix, including a highly saline matrix (NaCl up to 15%) and a relevant organic component, typically around 15%. Methods for trace element determination in this matrix have been scarcely reported and no procedure has been proposed including Total Reflection X-Ray Fluorescence (TXRF). Aim of the present paper is to setup a TXRF method requiring minimum sample treatment and suitable for process control and risk assessment related to soy sauce consumption.

Accordingly, a digestion (HNO_3/H_2O_2) plus dilution (1:5 w/w) procedure was developed, applied to six soy samples from the Chinese market and the results compared to Inductively Coupled Plasma–Mass Spectrometry data. As a result, the procedure was fully validated for the six elements that could be compared: Cu, Fe, Mn, Rb, Sr and Zn. A dilution only procedure was also assessed showing, on average, a −5% bias only. Accordingly, sample digestion yields highly accurate data, whereas a simple 1:5 dilution may be perfectly suited for most purposes. Regarding detection capabilities, the limits of detection are typically below 0.5 mg/kg for both digested + diluted and diluted only samples. The reported procedures are accordingly fit for purpose in quality assurance/quality control procedures and risk assessment related to soy sauce consumption.

1. Introduction

Soy sauce is a common condiment in East and Southeast Asia, possibly originating from China, now spread all over the world. The traditional recipe makes use of boiled soybean, roasted and crushed wheat with the addition of table salt. The mixture is fermented by fungi of the *Aspergillus* genus. Several different recipes exist, every country showing small or significant deviations from the abovementioned ingredients. Soy sauce is nowadays consumed worldwide although East and Southeast Asia lead by far the world market: just as a glimpse of the production scale, Chinese production of soy sauce was around 10 million tons in 2016 [1].

Interest in the characterization of this food commodity clearly stems from the extensive use of this dressing and seasoning agent, making a monitoring program desirable. Besides the general interest in monitoring, common to any largely diffused food, two specific cases should be mentioned as paradigmatic of the usefulness of analytical investigations on soy sauce samples. Regarding human health, undesired contamination in soy sauce may arise from several sources and has actually been detected in several studies (see [2] for the contamination by the carcinogenic compound 3-chloropropane-1,2-diol). Contamination by organic species is clearly outside the aims of the present research, but it highlights the relevance of surveillance studies on this product. The second case concerns the deliberate fortification of soy sauce by iron, mainly in the form of iron ethylenediaminetetraacetic acid (EDTA) complex. Being soy sauce extensively used in China, with consumption of up to 15 mL per day [3], the fortification of this dressing was introduced as an effective mean to fight anemia in the Chinese population [4]. Iron detection is clearly of the utmost importance to assess the efficacy of the fortification process.

From the analytical point of view, soy sauce is a complex matrix. Main components include 8% proteins, 5% carbohydrates, 0.8% fibre, 0.6% lipids, 5.5% sodium, 0.4% potassium [5]. It features both a non-negligible organic content and a distinctive salinity, features strongly complicating the detection of elements at the trace level. High viscosity and the presence of condensed phases (suspended particulate matter) should be additionally mentioned as physical factors further contributing to sample complexity. As a matter of fact, methods for trace element

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detection in soy sauce have been scarcely reported up to date (see e.g. [6] for an Inductively Coupled Plasma–Optical Emission Spectroscopy determination); moreover, as evidenced in a recent review [7] devoted to food analysis by Total Reflection X-Ray Fluorescence (TXRF), no application of this technique to soy sauce samples has been reported in the literature.

Aim of the present research is to assess the possibilities of TXRF to provide a rapid and reliable method for trace element determination in this important food commodity. Data on trace element concentrations in several commercially available soy sauce samples were determined and compared with results obtained by Inductively Coupled Plasma–Mass Spectrometry (ICP-MS). The fitness for purpose of the TXRF procedure will be discussed.

2. Materials and methods

2.1. Samples

Six different soy sauce were obtained from the Chinese market from six different producers and labelled soy1 to soy6. Samples differed in viscosity, composition (markedly salinity) and, possibly, production process (acidic hydrolysis vs. fungi fermentation).

2.2. TXRF procedures

In order to remove the organic fraction and to limit the mass deposited on the quartz reflector, a digestion in nitric acid and hydrogen peroxide followed by dilution was performed. One gram of each sample was transferred in a 30 mL Savillex perfluoroalkoxy alkane vial with a screw cap: gallium from a standard solution (Merck Gallium ICP standard in HNO_3 3%, 1000 mg/L) was spiked to have a concentration of 10 mg/kg in the final solution after dilution and used as the internal standard. Before closing the cap, 1.5 mL of HNO_3 (Merck pro analysis 65%) and 0.5 mL of H_2O_2 (Carlo Erba pure stabilized 40%) were added. After homogenization, digestion was performed by placing the vial on a hot plate at around 130 °C for 1 h. Finally, 1:5 dilution was achieved by adding ultrapure water (Carlo Erba Analytical Grade ≤0.1 μS/cm). The same procedure was performed in triplicate without adding any sample to assess the blank levels.

The accuracy of the above procedure was evaluated with a recovery test by adding 5 mg/L of a Merck ICP multi-element standard solution IV in 6% nitric acid, 1000 mg/L in Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Tl, Zn at. The same concentration for As was added separately from an arsenic ICP standard solution (Merck in 3% nitric acid, 1000 mg/L).

Besides digestion, a 1:5 dilution only with ultrapure water was attempted in order to investigate a possible fast way of quantifying elements of interest without any treatment, which may alter the sample composition. Instead of adding gallium, zinc was used as internal standard with a nominal concentration resulting from the corresponding digested samples.

For all the sample preparations described above, 8 μl were pipetted onto a siliconized (Serva Silicone Solution) quartz reflector, before drying at 90 °C on a hot plate. Three different reflectors were prepared for each sample.

2.2.1. Data acquisition and treatment

Measurements were collected using a benchtop TXRF spectrometer (Horizon, G.N.R. Italy), equipped with a monochromatic X-ray source (Mo-Kα, 17.4 keV) at 40 kV, 15 mA and a 25 mm^2 Silicon Drift Detector. Counting time for each specimen was 300 s, after correction for detector dead time.

Recorded spectra were analysed by the software supplied with the instrument: after background and spectrum identification and fitting, quantification was achieved through the Internal Standard method and relative sensitivity curves.

The Limit of Detection (LOD) for the analyte x is calculated as three times the standard deviation of background signal area N_{back} below its fluorescence peak of net area (intensity) N_x, according to Eq. (1) [8]:

$$LOD_x = \left(3c_x\sqrt{N_{back}}\right)/(N_x) \quad (1)$$

where c_x is the analyte concentration (mass fraction). The ratio between N_x and c_x is the Sensitivity factor.

2.3. ICP-MS procedure

Trace metals were determined by ICP-MS in the same samples by an iCAPQ instrument from Thermo Elemental. The latter was operated in the kinetic energy discrimination mode to enable iron detection on the 56 *m/z* channel: polyatomic interferences were reduced by introducing a 4.84 mL/min flux of helium in the collision cell. Further optimization of the instrumental parameters was performed to achieve best sensitivity, low levels of oxides (CeO^+/Ce^+ <2%) and of double charged ions (Ba^{++}/Ba^+ <3%). Mass calibration was regularly verified. Major instrumental parameters are as follows: plasma power 1.55 kW; gas flows: nebulizer 0.99 L/min, cool 14 L/min and auxiliary 0.8 L/min. Manufacturer suggested channels were acquired with a dwell time of 10 ms for element determination: five replicates per sample were measured (150 sweeps per replicate). Samples were simply diluted 1:500 (w/w) prior to analysis, adding concentrated pure nitric acid to a final concentration of 2% (pure HNO_3 was produced by a sub-boiling Milestone DuoPur apparatus). External linear calibration was used for analyte quantification in the range 0.1–75 μg/kg: standard solutions were obtained by diluting a Merck ICP multi-element standard VI.

3. Results and discussion

The assessment of the capabilities of TXRF to investigate this matrix followed two different paths. As a first consideration, no standard reference material exists for trace elements in soy sauce: validation strategies had to be adapted to this fact. Firstly, a recovery test was performed aiming at establishing the detection capabilities on a large number of elements, contained or not in the original samples. In the following Section 3.2, the concentrations of elements obtained after digestion with a HNO_3/H_2O_2 mixture were compared, whenever possible, to the ones obtained by the reference technique ICP-MS. Finally, a simple 1:5 dilution with no other added reagents was checked and the results compared to the ones obtained by the digestion+dilution procedure.

3.1. Recovery test

The recovery R for an analyte × of interest is given by Eq. (2) [9]:

$$R[\%] = (c_x - c_{x,0})/(c_{x,spike}) \cdot 100 \quad (2)$$

where c_x is the concentration of the analyte in the sample after spiking, $c_{x,0}$ its concentration before spiking and $c_{x,spike}$ the known added quantity.

Element recoveries were measured on all of the six digested soy samples and are reported in Fig. 1.

The recovery values are in the range 90%–110%. These figures are thus in compliance with the 80%–110% range set by Codex Alimentarius Commission Procedural Manual for the concentration range 1–10 mg/kg [10].

3.2. Sample treatment and validation by comparison with ICP-MS data

Sample treatment in TXRF is no less important than for any other analytical technique [11]. Specific to this technique is the well-known concept of critical thickness and critical mass, i.e. the sample physical conditions that warrant thin film approximation which translates into