Feasibility studies for new food matrix-Reference Materials

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Abstract. This paper reports about the feasibility studies for the development of new food matrix-Reference Materials (RMs) aimed to evaluate quality, safety and traceability of selected food products, performed during the "Early Phase" (H2020 INFRADEV-02-2016 PRO-METROFOOD, G.A. 739568) of METROFOOD-RI "Infrastructure for Promoting Metrology in Food and Nutrition" (ESFRI Domain "Health & Food"), in the frame of the development of pilot services. With this purpose, batches of oyster tissue, rice grains and rice flour were prepared in lyophilized form (oyster tissue) or in their raw form (rice), homogenised, partitioned and then characterised for a very wide set of parameters, such as: contaminants, biomarkers, stable isotopes, markers of origin/authenticity. Such a wide characterisation was aimed to involve as many laboratories as possible and to go forward the development of "*Multipurpose*-RMs", meaning new RMs having certified, reference or information values for several different parameters. Homogeneity studies and stability studies under thermal and luminous stress are underway.

1. Introduction

In food analysis, RMs play an important role in adopting metrological concepts and in many cases represent the only way to guarantee the quality of chemical and biological measurements by allowing to establish metrological traceability of the measurement results and quantify measurement uncertainty. They can be used for method validation and performance verification, instrumental calibration, evaluation of measurement uncertainty, quality control, etc. Despite an increase in the production of new RMs in recent years, there is still a lack of fit-for-purpose RMs especially for the agrofood sector and there is a continuous need to develop new RMs with different matrix/analyte combinations to cover analytical requirements. This need is related to many factors, such as the increasing innovation in analytical techniques, the development of new analytical methods able to detect/determine new parameters of emerging interest, the development of new methods related to food profiling (for quality, authenticity, traceability), and the need to study contaminants of emerging concern [1]. Within the PRO-METROFOOD project (G.A. 739568), pilot services have been organized to test the inter-operability and to demonstrate the actual capability of METROFOOD-RI (www.metrofood.eu) to deliver services providing an added value as organized Research Infrastructure (ESFRI Domain "Health & Food). Considering that one of the "core services" offered by METROFOOD-RI is the development and provision of new (customized) RMs, feasibility studies dedicated to the development of new RMs of food matrixes were carried out. They included: RM preparation; definition of the procedures and guidelines to collect the characterisation results and to process obtained data; RM characterisation; homogeneity and stability studies; data processing and result evaluation. With this purpose, according to BCR and ISO Guides [2,3], feasibility studies for the preparation of new Matrix-RMs of rice grains, rice flour and lyophilised oyster tissue were conducted. As further added value, a very wide characterisation of the materials was performed, in order to promote the development of *Multipurpose*-RMs, accompanied by certified, reference or information value for many different parameters. In fact, the possibility to prepare *Multipurpose*-RMs, intended for use in multi-parametric determinations, qualitative analyses and identity studies, through the definition of elemental, molecular and/or genetic markers or patterns for traceability of food products, looks particularly interesting [4,5,6]. RMs to be developed were selected based on: current RM availability, gaps in current production, and main analytical challenges related to the selected matrixes. Matrices were selected also with the aim to represent cereals as staple food of worldwide relevance and a food matrix of animal origin known to accumulate a huge variety of contaminants and residues (oysters). Further, these matrices are important for verifying authenticity of adulterated products.

2. Reference Material preparation

The preparation of different batches of candidate RMs was performed by ENEA.

Two batches of rice grains and rice flour RMs were prepared starting from lots of about 80 kg of super-fine Carnaroli white rice grains and super-fine Carnaroli white rice flour (same variety and same origin) provided by Ente Nazionale Risi (Italy). Raw materials were at first homogenized in a Vrieco Nauta® Conical Mixer (V = 240 l) equipped with a dosing valve, with the following order of processing: 1) rice grains; 2) rice flour. After grain homogenization, the dust remaining on the Nauta inner walls was removed by blowing N_2. Both for grains and flour, the Nauta was at first rinsed with an aliquot (about 5 kg) of the material to be processed; rinsing was carried out by rotating the Nauta for 15 min, collecting and reloading multiple times the same material. The material used for rinsing was then discarded. The Nauta was then loaded with all the available material (75 kg) and allowed to rotate for 2 hours. Aliquots (3.2 kg) were then collected in plastic bags using the dosing valve, always maintaining rotation. All the materials were checked for free water (a_w) at the beginning and at the end of the procedure. The bags were sealed under vacuum and then shipped to the Casaccia Research Centre. On arrival, the material contained in each bag was re-homogenised and partitioned using a Retsch sample divider (8 sub-aliquots) by applying a two-step procedure. Finally, the material was bottled in plastic bottles and labelled, realising final aliquots of 80 g for rice grains and 50 g for rice flour.

One batch of oyster tissue-RM was prepared starting from a 60 kg lot of frozen un-shelled oysters provided by UPPA (France). The oysters were originally collected in the Bay of Arcachon and in the Bay of Oléron to assess series of contaminants of use for the European Water FrameWork Directive. Raw materials were first partially defrosted, milled and homogenized in a miller equipped with anti-contamination blades, preliminarily rinsed with an aliquot of the material to be processed (the material used for rinsing was discarged). Then, milled oysters were lyophilised using a Virtis Advantage system (Tmin sample = -35°C, Tmax sample = +30°C, p min = 50 mtor); totally, 4 lyophilisation cycles were performed. After lyophilisation, the material was checked for free water (a_w < 0,07 at Tamb. for all checked samples for each lyophilisation cycle). Lyophilised aliquots (3.2 kg) were then sieved to verify granulometry and checked for any eventual un-wanted residue of shells, and then collected in plastic bags sealed under vacuum, which were shipped to the Casaccia Research Centre. On arrival, the material contained in all bags was re-homogenised in a Vrieco Nauta® Conical Mixer (V = 20 l) and then partitioned using a Retsch sample divider (8 sub-aliquots). Finally, the material was bottled in plastic bottles and labelled, realising final aliquots of 25 g.
Figure 1 shows the block diagrams of the RM preparation procedures.

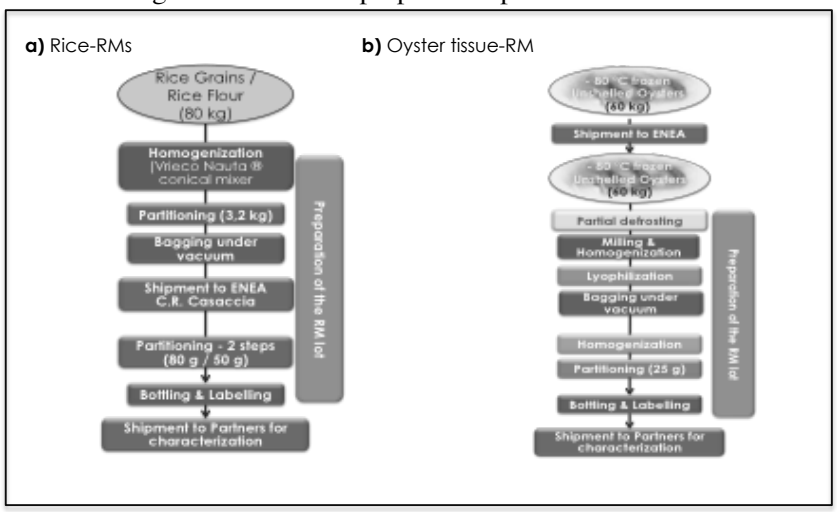

Figure 1. Diagram blocks for the RM preparation

3. Interlaboratory study

For material shipment, each bottle was put in a plastic bag sealed with silica gel inside, and then boxes containing 5 bottles of RM plus 1 blank bottle were prepared, closed and further sealed with plastic film. The materials were shipped to 39 laboratories for characterization in the following Countries: Belgium, Czech Republic, FYROM, France, Germany, Greece, Hungary, Italy, Moldova, Portugal, Romania, Slovenia, and Turkey. The shipment was accompanied by a document of "Guidance for characterization of Reference Materials" providing instructions for material storage and handling. It was recommended to store the materials at room temperature (T max = 25 °C) in the dark and in a low-humidity environment. Instructions were provided also to determine moisture content, using the blank bottle. Furthermore, instructions and dedicated spreadsheets were provided for result analysis and submission. Each laboratory was assigned an ID number and the data collection was organized to guarantee the anonymity of the participating laboratories during statistical analysis and data evaluation. To provide a wider set of parameters, each laboratory involved in the inter-laboratory comparison exercise has first identified its own analytical capability and expertise for RM characterization, considering different classes of parameters. Finally, the following parameters were included in the characterisation:

- Nutrients and bioactive compounds - Carbohydrates, Fibers (total, crude, dietetic; amylose content), Mineral salts (Ca, K, Na, Mg, P) and trace elements, Vitamins (C, B group), lipids, fatty acids, protein fractions, aminoacids, phenols, tocols, flavonoids and flavonols

- Bioactivity and reactivity – Antioxidant activity
- Organic Contaminants and Residues - Toxigenic fungi and toxins; pesticides, pharmaceutical residues and veterinary drugs
- Inorganic Contaminants - Toxic elements (e.g.: As, Cd, Cu, Hg, Ni, Pb); Speciation (As speciation, Hg and MeHg, Sn and organotin compounds)
- Stable isotopes (e.g.: Hg and Sr isotopes; stable isotopes of light elements e.g. C, N, S)
- Microbiological analysis
- Physical characterization (e.g.: ash, particle size, gelatinization-retrogradation, texture parameter of cooked rice)
- Allergens profile characterization
- Genetic analyses - Rice endogenous/GM construct screening

Parameters were selected based on the possibility to involve in the characterisation as many Labs. of the Consortium as possible and perform cross-comparisons, with the final purpose to test and demonstrate the inter-operability of the infrastructure. All results reported by participants have been statistically evaluated using standard procedures and methods for evaluation. Results have been obtained for the different parameters and are now in processing. While for some parameters (e.g. toxic elements, mineral salts) many laboratories participated to the interlaboratory study and it is possible to move forward certification, in some other cases only a few number of results are available and therefore it is possible to perform only general evaluation and provide reference values. Concerning mycotoxins, for example rice grains and rice flour were characterised for the following mycotoxins: DON, 15-ADON, 3-ADON; AF B1, B2, G1, G2; Beauvericin; Citrinin; Diacetoscirpenol; Enniatin A, A1, B; Fumonisin B1, B2, B3; FUSARENONE X; T2, H-T2 toxin; NIV; OTA; Alternariol; Tentoxin; ZEA, α-ZEA, β-ZEA. In almost all cases, analysed mycotoxins resulted < DL and the LoQ indicated by the involved laboratories were compared with the maximum levels foreseen by the Reg. (EC) No 1881/2006 (cons. 07/17). Homogeneity studies have been carried out and the results are being processed. In general, for the processed data, the consortium showed to provide consistent, comparable results despite using different pre-treatment procedures and analytical methods. Furthermore, isochronous stability tests are being carried out under both thermal (at different temperatures) and luminous stress on all the materials.

4. References

[1] M Rychlik, et al (2018). Ensuring Food Integrity by Metrology and FAIR Data Principles, Front. Chem. 6:49. doi: 10.3389/fchem.2018.00049
[2] Guidelines for the production and certification of BCR reference materials. Doc.BCR/01/97 – Part A, 1997.
[3] ISO GUIDE 35:2017. Reference materials - Guidance for characterization and assessment of homogeneity and stability.
[4] G Zappa, C Zoani. Reference Materials in support to Food Traceability. “Frontiers in Food Science for Feeding the World” Agrochimica - Pisa University Press [ISSN-IT0002-1857]
[5] G Zappa et al. Feasibility Study for the Development of a Honey-Reference Material. 16th Int. Congress of Metrology, 16001 (2013) DOI: 10.1051/metrology/201316001.
[6] C Zoani et al. Development of innovative Reference Materials for the agrofood sector. Int.Conf. 1st IMEKOFOODS Rome,12-15/10/ 2014

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