

Intra and inter laboratory reference materials for multi element stable isotope analysis in food authentication

Position Paper of the Working Group Stable Isotope Analysis

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Stable isotope analyses of H, C and O have been used for food authenticity control since 1990. Official methods for wine stable isotope analyses were introduced in the EU in 1990, and for products as fruit juices, honeys, maple syrup in the EU and the US between 1990 and 2000. More recently authenticity checks, which refer to the “geographical authenticity” mainly of premium food products, with protected denomination of origin (PDO), were developed. The determination of one or two stable isotope parameters, as applied to detect addition of water or sugars, usually is not sufficient to obtain unambiguous results regarding geographical origin. Evidence of work, including EU project Tracing Food Commodities in Europe (TRACE), strongly supports the use of multi element stable isotope methods, which means combination of stable isotope data of all “bio-elements” (H, C, N, O, S), and sometimes of “geo-elements”, as strontium, in addition.

One prerequisite for application of multi element stable isotope methods to origin assignment, not only for food products, but even in forensic or ecological investigations, is the availability of accepted reference materials, or ideally, certified reference materials, containing all relevant elements in an elemental composition similar to the samples investigated. In fact there is still a lack of official reference materials (as supplied by International Atomic Energy Agency (IAEA), Community Bureau of Reference (BCR), or United States Geological Survey (USGS) which fit that description, most of them are either mineral substances (e.g. nitrate, sulphate), or they do not contain all bio elements, or if so, not in an elemental composition as required (Brand et al., 2014, Schimmelmann et al., 2016, Camin et al., 2017). In addition, official reference materials are available only in small quantities (a few g) and at relatively high prices (several hundred €), which prevents their use in daily batch analyses as demanded or suggested recently (Brand et al., 2014). Several inter comparison substances which meet the requirements (e.g. casein, collagen, wheat flour) have been characterised during TRACE or even in earlier EU projects, but they are not or not longer available in sufficient amounts, and they have neither any acknowledgement nor certification.

For the required use of intra and inter laboratory reference materials, to cover the range of usual isotopic data for all bio elements and the elemental composition of the routine samples, at least two protein samples and two samples of plant materials like flour should be available. The main difference between proteins and plant materials (as flour) is the different elemental content of nitrogen and sulphur (protein about 12 % nitrogen and about 0.5 to 1 % of sulphur, plant material about 2 % of nitrogen and < 0.1 % of sulphur). In addition, the amount of exchangeable hydrogen is quite different for proteins like casein (about 15 % exchangeable hydrogen) and plant material (about 40-50 % exchangeable hydrogen). Considering the influence of high nitrogen and sulphur content on the hydrogen isotope analysis by high temperature conversion (HTC), and probably on oxygen isotope determination using HTC as well, these are further reasons for the use of reference materials which are as similar as possible to our routine samples.

To deal with those problems, the working group stable isotope analytics of GDCh (AG Stabilisotopenanalytik) decided to supply and characterize stable isotope data for such materials by repeated inter laboratory analyses in the member laboratories and to fix stable isotope data for such materials by agreement. As a first practical attempt 10 kg of casein (produced as a painting pigment) from a German supplier in Bavaria (Kremer Pigmente GmbH & Co.KG, 88317 Aichstetten) were bought and distributed to the stable isotope laboratories involved in the working group. This material herein referred as “Casein Iso 1” has been analysed in inter laboratory

exercises called “KPT” (Kleiner Proficiency Test) from January 2015 to February 2017 in 8-12 European stable isotope laboratories (Table 1), which are all accredited according to DIN EN ISO/IEC 17025:2005 and have traced back their intra laboratory calibration to the official stable isotope reference materials as being distributed by IAEA Vienna. The elemental composition of this casein sample is about 8 % hydrogen, 14 % nitrogen, 49 % carbon, 28 % oxygen, and 0.6 % sulphur.

Table 1 List of participating stable isotope laboratories

Institution	Country
Agroisolab GmbH	Jülich, Germany
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit	Oberschleißheim, Germany
Bundesinstitut für Risikobewertung	Berlin, Germany
Chemisches und Veterinäruntersuchungsamt	Freiburg, Germany
Chemisches und Veterinäruntersuchungsamt Münsterland-Emscher-Lippe AöR	Münster, Germany
Eurofins Analytics	Nantes, France
Institut des sciences analytiques	Villeurbanne, France
isolab GmbH Laboratorium für Stabile Isotope	Schweitenkirchen, Germany
Lund University	Lund, Sweden
Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit	Oldenburg, Germany
Fondazione E. Mach - Istituto Agrario di San Michele all'Adige	San Michele all'Adige, Italy
The Food and Environment Research Agency	York, United Kingdom
Universität Hohenheim, Institut für Lebensmittelchemie	Hohenheim, Germany

The laboratories were asked to process this sample as they do with their routine samples, too, and to perform multi element stable isotope analyses as far as they are able to do. Some of the laboratories only determined carbon and nitrogen isotopic ratios, others measured all elements from hydrogen to sulphur. The laboratories applied different analytical systems (Thermo Fisher, Isoprime/Elementar, PDZ Europa, Hekatech, Nu instruments) and from that different methodology

e.g. with regard to combustion/high temperature conversion reactor filling, combustion/conversion temperatures and gas separation system.

The results of those analyses have been compiled and preliminary stable isotope data for this sample can now be suggested by agreement between participating laboratories. The results obtained are as given below (table 2) and the suggested stable isotope data are as indicated in the last row.

Table 2 $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ values of Casein Iso 1 of different KPT rounds

Round	$\delta^2\text{H}$ [‰] vs. V-SMOW		$\delta^{18}\text{O}$ [‰] vs. V-SMOW		$\delta^{13}\text{C}$ [‰] vs. V-PDB		$\delta^{15}\text{N}$ [‰] vs. Air		$\delta^{34}\text{S}$ [‰] vs. V-CDT	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	-114.7	2.9	11.9	0.7	-25.5	0.1	6.3	0.3	5.9	0.3
2	-113.4	2.8	11.5	0.9	-25.4	0.4	6.3	0.1	5.9	0.5
3	-111.6	1.5	12.2	2.0	-25.5	0.1	6.4	0.6	6.1	0.3
4	-110.2	2.6	12.2	2.1	-25.5	0.1	6.4	0.2	5.6	0.7
5	-113.3	3.3	12.0	1.8	-25.6	0.1	6.3	0.2	5.8	0.5
6	-112.6	3.0	10.9	3.5	-25.5	0.1	6.3	0.4	5.8	0.4
7	-111.8	1.2	11.5	2.0	-25.1	0.1	6.3	0.1	5.7	0.4
8	-111.7	2.6	12.1	1.4	-25.5	0.1	6.3	0.1	5.6	0.6
Summary	-112.7	2.8	11.7	1.6	-25.5	0.2	6.3	0.3	5.8	0.5
Total N	46		27		83		82		40	

As expected, the number of results was the highest for carbon and nitrogen isotopic ratios (83 and 82 individual results from up to 11 laboratories per round), for hydrogen and sulphur isotopic data we have 46 and 40 individual results from 5 or 6 laboratories, and for oxygen isotopic ratios the number of results is only 27 individual data from 3 or 4 laboratories. The variability of the results between laboratories is quite acceptable for carbon and nitrogen values (exception of round 2 for carbon and round 3 for nitrogen). The higher variability in some cases is due to outlying results from one laboratory which have not been removed, but this does not remarkably affect the mean values for all participants. In case of hydrogen and sulphur isotopes, the number of individual results is only half of those for carbon and nitrogen isotopic data, there are more rounds with one or two results that deviate remarkably from the mean values (rounds 1, 2, 3, 5, 7 for hydrogen, rounds 5 and 7 for sulphur), but the mean values still indicate acceptable agreement between different KPT rounds, and the total mean values for those elements can be considered as reliable, too. The situation for oxygen isotope data of casein is still not satisfying, that may be due to a not sufficient number of participating laboratories and/or the fact that calibration (international reference materials applied) and design of the individual high temperature conversion systems are very different between laboratories (the conversion temperature is different and some laboratories apply a simultaneous H and O isotope analysis while others run

both elements separately). The effects of modifications of the HTC system for conversion of certain organic materials have already been described elsewhere (Sieper et al., 2010, Qi et al. 2011, Gehre et al., 2017).

To proceed, our aim is to apply the same procedures to another casein material which should possess stable isotope data as different as possible from our present sample. This requires to have a sample with very high ^{13}C content (from South America or Australia?), a very low or very high nitrogen and sulphur isotopic ratio, and high hydrogen and oxygen isotopic values. Further aims could be to provide and analyse wheat or rice and maize flour samples to have two isotopically different materials as representatives of plant material as well.

Literature

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