

## REFERENCE MATERIALS AND CALIBRATION FOR STABLE ISOTOPE RATIO ANALYSIS IN FOOD

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**Abstract** – The stable isotope ratios of the five bio elements carbon, nitrogen, hydrogen, oxygen and sulphur have frequently been used in the last few decades to determine the authenticity and traceability of many food commodities. In this paper, issues regarding calibration, suitable reference materials and correct methods to obtain reliable results with this potentially powerful technique are critically discussed and commented.

**Keywords:** stable isotope ratios, IRMS, reference materials, calibration

agreed standards ( $R_{REF}$ ): Vienna - Standard Mean Ocean Water (V-SMOW) for  $\delta^2H$  and  $\delta^{18}O$ , Vienna –

Pee Dee Belemnite (V-PDB) for  $\delta^{13}C$ , air for  $\delta^{15}N$  and Vienna-Canyon Diablo Troilite (V-CDT) for  $\delta^{34}S$  [4].

Despite widespread use of SIRs and official use of this technique in the last few years in specific cases, some issues related to reference materials and the calibration of SIRA have been highlighted. The aim of this paper is to give an overview of these issues and the proposed solutions.

### 1. INTRODUCTION

Stable isotope analysis has been used to check food authenticity and traceability for more than 30 years for a wide variety of food commodities. As a consequence, today this technique is also utilised on a routine basis as an official method (Table 1) e.g. to verify the authenticity of wine, fruit juice, olive oil and honey [1, 2, 3].

The use of stable isotope analysis for food authentication is based on the fact that several processes and reactions (biological, biochemical, physical, chemical etc.) fractionate individual isotopes of an element and consequently generate unique isotopic signatures.

Stable isotope compositions are normally reported as delta values ( $\delta$ ) and presented in units of parts per mill (‰) according to the equation (1):

$$\delta i E = \frac{(i R_{SA} - i R_{REF})}{i R_{REF}} \quad (1)$$

$R_{SA}$  and  $R_{REF}$  are the molar ratios of the heavy isotope relative to the light isotope, in the sample and standard respectively. The values are normally reported relative to the following internationally

### 2. DESCRIPTION OF THE CONTRIBUTION

The first applications of stable isotope ratio analysis concerned 'environmental' sciences such as geology, hydrology, palaeoclimatology and archaeology. For this reason the primary reference materials and anchoring points derived from geology (i.e. PDB – Pee Dee Belemnite carbonate, SMOW – Standard Mean Ocean Water, CDT – Canyon Diablo Troilite, Nitrogen – air nitrogen), like most of the reference materials developed over the years. For this reason the first issue arising as regards this type of technique applied to food is that at the moment, well-calibrated isotopic reference materials for all types of sample compounds and matrices are not available (see for example the list of reference materials that currently have certified values on the IAEA website, Table 1).

SIRA is ideally conducted using reference materials that have chemical and physical properties, including isotopic composition, similar to those of the measured samples. Furthermore, samples and standards should be processed in the same manner during preparation and analysis (Identical Treatment principle) [4], in order to avoid

different isotopic fractionation and/or contamination. This problem is particularly important for  $\delta^{15}\text{N}$  determination, as organic and inorganic compounds behave differently when analysed using EA-IRMS. In particular, this is related to the tailing of the nitrogen leak of nitrate-containing compounds [5] and leads

Table 1. List of the current stable isotope ratio standards currently have certified values on the IAEA website.

	Matrix	$\delta^2\text{H}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$
VSMOW2	Water	0±0.3		0±0.02		
SLAP2	Water	-427.5±0.4		-55.5±0.02		
IAEA-604	Enriched Water	799.9±0.4		-5.86±0.04		
IAEA-605	Enriched Water	5997.9±0.5		-3.02±0.05		
IAEA-606	Enriched Water	15993.6±1.0		2.43±0.04		
IAEA-607	Enriched Water	802.4±0.4		99.02±0.13		
IAEA-608	Enriched Water	6014.3±0.5		736.4±0.9		
IAEA-609	Enriched Water	16036.±1		1963.7±2.2		
IAEA-N-1	Ammonium Sulfate				+0.4±0.2	
IAEA-N-2	Ammonium Sulfate				+20.3±0.2	
IAEA-NO-3	Potassium Nitrate			+25.6±0.4	+4.7 ± 0.2	
USGS32	Potassium Nitrate			+25.7 ±0.4	+180 ± 1	
USGS35	Sodium Nitrate			+57.5±0.6	+2.7± 0.2	
USGS25	Ammonium Sulfate				-30.4± 0.4	
USGS26	Ammonium Sulfate				+53.7± 0.4	
NSVEC	Nitrogen Gas-N <sub>2</sub>				-2.8± 0.2	
IAEA-305	<sup>15</sup> N labelled ammonium sulfate				(vial A) 39.8 (vial B) 375.3	
IAEA-310	<sup>15</sup> N labelled urea				(vial A) 47.2 (vial B) 244.6	
IAEA-311	N-labelled ammonium sulphate				+2.05	
IAEA-S-1	Silver Sulfide					-0.3
IAEA-S-2	Silver Sulfide					+22.7±0.2
IAEA-S-3	Silver Sulfide					-32.3±0.2
IAEA-S-4	Sulfur					+16.9±0.2
IAEA-SO-5	Barium Sulfate					+0.5±0.2
IAEA-SO-6	Barium Sulfate					-34.1±0.2
NBS-127	Barium Sulfate			+9.3±0.4		+20.3±0.4
NBS 22	Oil	-120 ±1	-30.031 ±0.043			
NBS 28	Quarz Sand			+9.6 ±0.1		
USGS24	Graphite		-16.049±0.035			
IAEA-600	Caffeine		-27.771± 0.043		+1.0±0.2	
IAEA-601	Benzoic Acid			+23.3±0.3		
IAEA-602	Benzoic Acid			+71.4±0.5		
IAEA-CH-3	Cellulose		-24.724± 0.041			
IAEA-CH-7	Polyethylene	-100.3± 2.0	-32.151± 0.050			
IAEA-303	<sup>13</sup> C Labelled Sodium-Bicarbonate		(Amp. A) 93.3 (Amp. B) 466			

to the application of different thermochemistry to the two types of compounds. For this reason international secondary inorganic reference materials should not be used for calibration during the analysis of organic compounds. The same situation occurs for  $\delta^{34}\text{S}$ . In general, because there are no suitable reference materials applicable for multi-isotopic analysis in all food matrices at the

moment, working standards are normally used in routine laboratory tests, if possible with the same matrix as the samples. These are calibrated against primary or secondary reference materials and used in each sample sequence. This could lead to problems in the assignment of the 'true' values of the working standards, despite significantly improving the time and costs associated with such analysis.

Lately, researchers have tried to remedy this situation and some organic reference materials with recommended values have been placed on the market (i.e. USGS – Reston Stable Isotope

Laboratory, <http://isotopes.usgs.gov/lab/referencematerials.html>).

Table 2. List of the current stable isotope ratio standard methods usable for verifying the declared geographical origin of food.

Method	Product	Stable isotope ratio	Instrument	Databank	References for databank
OIV-MA-AS311-05	wine, must	(D/H), and (D/H) <sub>II</sub> of ethanol	<sup>2</sup> H-SNIF-NMR	EU wine databank	EU Reg 555/2008
OIV-MA-AS312-07	wine, must	<sup>13</sup> C/ <sup>12</sup> C of ethanol	IRMS	EU wine databank	EU Reg 555/2008
OIV-MA-AS2-12	wine, must	<sup>18</sup> O/ <sup>16</sup> O of water	IRMS	EU wine databank	EU Reg 555/2008
ENV 12140	fruit juice	<sup>13</sup> C/ <sup>12</sup> C of sugar	IRMS	AIJN, literature	[6, 7, 8, 9]
ENV 13070	fruit juice	<sup>13</sup> C/ <sup>12</sup> C of pulp	IRMS	AIJN, literature	
ENV 12140	fruit juice	<sup>18</sup> O/ <sup>16</sup> O of water	IRMS	AIJN, literature	
AOAC 995,17	fruit juice	(D/H), and (D/H) <sub>II</sub> of ethanol	IRMS	AIJN, literature	
AOAC 2004.01	fruit juice	<sup>13</sup> C/ <sup>12</sup> C of ethanol	IRMS	AIJN, literature	
[10]	cheese	<sup>13</sup> C/ <sup>12</sup> C, <sup>2</sup> H/ <sup>1</sup> H, <sup>15</sup> N/ <sup>14</sup> N, <sup>34</sup> S/ <sup>32</sup> S of defatted cheese	IRMS	PDO Grana Padano and Parmigiano Reggiano databank	EU Regulation 584/2011
[11]	olive oil	<sup>13</sup> C/ <sup>12</sup> C, <sup>2</sup> H/ <sup>1</sup> H, <sup>18</sup> O/ <sup>16</sup> O of bulk olive oil	IRMS	Italian PDO databank	Submitted to IOC

OIV: International Organization of Vine and Wine; ENV-CEN: European Committee for Standardization; AIJN: EUROPEAN FRUIT JUICE ASSOCIATION; AOAC: Association of Official Analytical Chemists; IOC: International Olive Council

A problem that can affect  $\delta^{18}\text{O}$  determination is the fact that the use of insufficiently long GC columns can lead to overlapping of the N<sub>2</sub> peak with the subsequent CO peak. For this reason, control of peak separation must be carried out and in the event of overlapping, longer columns must be used.

The lack of certified standards is particularly problematic for  $\delta^2\text{H}$  analysis of non-water samples, for which only a few reference materials exist at the moment, including only two organic reference materials with exchangeable H atoms. Indeed, at the moment  $\delta^2\text{H}$  analysis using IRMS is more challenging because of the possible presence of H atoms in exchangeable positions (i.e. amino, carboxyl, hydroxyl and thiol groups). Exchangeable H atoms must be taken into account, as they could exchange

with atmospheric water vapour, leading to potentially erroneous results if not considered. A number of methods have been developed to overcome this problem. Some examples are the comparative equilibration technique [12], standard ambient temperature equilibration [13], as well as steam equilibration [14], but the debate on this issue is still ongoing [15] and lately new technological solutions have also been proposed [16]. Furthermore, for both  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  analysis, it is important to dry samples before analysis, particularly in the case of hygroscopic materials, in order to eliminate any residual moisture.

In the case of  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ , the IUPAC guidelines recommend using scales normalised adopting linear regression with two anchoring points

(two-point normalisation) [17], if possible covering the entire range of  $\delta$  values typically observed in nature, in order to have a more robust method. This type of normalisation makes it possible to avoid scale compression or expansion that can lead to substantial deviation of the measured values. In particular, IUPAC has established that  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values must be expressed in relation to the VSMOW - SLAP scale (assigned values 0‰  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  for VSMOW and -428 ‰  $\delta^2\text{H}$  and -55.5‰  $\delta^{18}\text{O}$  for SLAP), whereas  $\delta^{13}\text{C}$  values must be expressed in relation to the VPDB scale, normalised by assigning the consensus values of -46.6 ‰ to LSVEC lithium carbonate and +1.95 ‰ to NBS 19 calcium carbonate [4].

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### 3. CONCLUSIONS

Over the years, stable isotope ratio analysis has been shown to be a powerful technique when applied to food traceability and authenticity, but some issues concerning calibration and reference materials have arisen. For this reason, it is highly important that published studies report all the details in relation to the method applied, in order to allow comparison of the results with other studies. Furthermore, there is currently an urgent need to develop new international reference materials covering all food matrices, as well as all isotopic ratios.

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