

## Stable isotope databases for European food products

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### Abstract

Stable isotope ratio analysis (SIRA) of the so-called bioelements (H,C,N,O,S) and the heavy element strontium is applied for food authenticity control and for geographical origin assignment of food products since about 30 years. The first official methods eg for honey and fruit juices have been acknowledged in 1978 and 1981 by AOAC. In 1990 the EU decided to install an official database for wine stable isotope parameters from all wine producing member states, at the same time accepting the <sup>2</sup>H-NMR (Nuclear Magnetic Resonance Spectroscopy) analysis of wine ethanol as official method. This database now contains the stable isotope data for hydrogen, carbon and oxygen (measured by IRMS= Isotope Ratio Mass Spectrometry) from wine ethanol and wine water of about 1000 authentic samples per year, which have now been collected for 17 years from several countries.

The same stable isotope methods as for wine are applied for fruit juices as well, therefore the Schutzgemeinschaft der Fruchtsaftindustrie (SGF) e.V. in Nieder-Olm compiled a fruit juice database since 1991, which has been further extended during an EU project ("PURE JUICE") from 2002-2005 and is now accessible via internet by the members of SGF, PURE JUICE participants and public control laboratories ([www.purejuice.org](http://www.purejuice.org)). The AG Stabilisotopenanalytik of the GDCh has recently installed a stable isotope database for fruit juices as well ([www.agstabilisotopen.org](http://www.agstabilisotopen.org)), which should be extended to other products, but is at present only accessible for members of this group.

In a recent EU project "TRACE" ([www.trace.eu.org](http://www.trace.eu.org)) stable isotope data are collected for a variety of food commodities, as honey, cereals, lamb, beef, olive oil, and mineral water. At the same time surface water and soil samples are taken and their stable isotope (and other) parameters analysed as well.

The aim of this project is to model and finally to predict stable isotope data for food commodities from the geographical, climatic, geological, botanical and hydrological information available even for regions where no authentic samples are accessible. If this works, the efforts for compiling databases of stable isotope data could be remarkably reduced.

In case of wood stable isotope data for several regions have been collected since a long time by climatologic researchers, as they use hydrogen and oxygen isotopes of tree ring cellulose to obtain information about the climate of the past. The substance analysed usually is pure  $\alpha$ -cellulose, which has to be prepared from raw wood by laborious preparation methods. A recent paper has indicated that it might be possible to replace this procedure by a less time consuming method applying the hydrogen isotope ratio of methyl groups instead of the cellulose. Finally, it should be mentioned that hydrological research has produced data collections of water stable isotope data (precipitation and ground water) from many regions and over about 40 years, especially in Europe, but for other regions of the world, too.

The information gathered in those databases could be very useful for geographical origin assignment of wood sample.

### 1 Basic information about stable isotope analysis

Chemical elements occur in nature as mixtures of two or more stable isotopes. Especially for the light elements H,C,N,O and S, the so called bio elements, as they form the basis of all living matter, the light isotope is by far more abundant as the heavy isotope(s). In case of carbon, the isotope <sup>12</sup>C has about 98.9 %, the heavier isotope <sup>13</sup>C about 1.1 % natural abundance. The mean natural isotopic ratio (eg <sup>13</sup>C/<sup>12</sup>C) of these bio elements is not absolutely constant, it shows distinct and systematic variations due to physical and biochemical isotopic fractionation, which can be used to conclude about the history and provenance of biochemical and/or biological material from different sources, even if it is chemically pure and/or identical (Kelly et al., 2005, Rossmann 2001). This possibility has been used for questions of geology, archaeology, forensics, environmental sciences and food authenticity.

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As the absolute isotopic ratio are difficult to handle in practise, stable isotope data are generally used as “delta-values”, which are the deviation of the stable isotope ratio of a substance from that of the commonly accepted reference material, as e.g. ocean water for hydrogen and oxygen isotopes or air nitrogen for nitrogen isotopes. In the same way, some heavier elements,

which have been applied in geological sciences for dating purpose, as one of their isotopes is formed by radioactive decay (so called geo elements), can provide complementary information especially for geographical origin assignment of food commodities (Hölzl et al. 2004, Horn et al. 1998, Kelly et al. 2005).

Table 1:

Elements and their stable isotopes, which are important as components of living matter (bioelements), their relative mean natural abundance F, the name of the International Isotopic Standard and its isotopic ratio R for the two most abundant stable isotopes.

$F = \text{isotope} / \text{Sum isotope}$ .  $R = \text{isotope a} / \text{isotope b}$

For oxygen and sulfur there are in addition a third or a third and a fourth, but less abundant stable isotope, from which the isotopic ratio(s) is (are) usually not applied for analytical purpose (Hoefs, 1981; Winkler and Schmidt, 1980)

Element	symbol	Isotop F [atom-%]	standard name	R
hydrogen	<sup>1</sup> H	99.9855	Standard Mean Ocean Water (=SMOW)	0.00015576
	<sup>2</sup> H=D	0.0145		
carbon	<sup>12</sup> C	98.892	Pee Dee Belemnite (=PDB)	0.011237
	<sup>13</sup> C	1.108		
nitrogen	<sup>14</sup> N	99.6337	Air (Air nitrogen) (=AIR)	0.0036765
	<sup>15</sup> N	0.3663		
oxygen	<sup>16</sup> O	99.7587	Standard Mean Ocean Water (=SMOW)	0.00200520
	<sup>17</sup> O	0.0375		
	<sup>18</sup> O	0.2039		
sulfur	<sup>32</sup> S	95.018	Canyon Diablo Troilite (=CDT)	0.0450045
	<sup>33</sup> S	0.750		
	<sup>34</sup> S	4.215		
	<sup>36</sup> S	0.02		

$$\delta\text{-value} = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \text{ [‰]}$$

## 2 Some examples for application of stable isotope data banks in food control

Stable isotope analysis of the light elements, especially of hydrogen, carbon and oxygen isotopic ratios is now applied for food authenticity control since more than 20 years. The aims of those investigations are preferably to check if products as wine, fruit juices, honey or spirits had been extended or adulterated by illegal addition of water and/or sugars (Bauer-Christoph

et al. 1997, Christoph et al. 2004, Engel et al. 2007, Kelly et al. 2005, Rossmann 2001). For this purpose several official methods have been adopted by European or US authorities or institutions (EU Commission, CEN, AOAC).

One of the well-known fields of stable isotope work is the European stable isotope Wine Databank and the application of the <sup>2</sup>H-NMR analysis of wine ethanol and the <sup>13</sup>C and <sup>18</sup>O IRMS analysis of wine ethanol and wine water,

respectively, for the control of sugar addition and water addition in wines (Christoph et al.2004). For stable isotope methods used in food authenticity control it is necessary to have a database of results for authentic samples available, which can be used for comparison of data from commercial products with them. That

wine databank is built since 1990 for all wine growing EU countries, and a number of about 1600 authentic samples is added to this databank per year. The total number of currently more than 15000 datasets is collected and stored at the JRC of the EU in Ispra (Italy).

Table 2:

Elements and their stable isotopes, which are important in Geology for dating purpose and which can be used for geographical origin assignment of foodstuff, their mother nuclides (radioactive), half-life times and natural abundance ratios R for the radiogenic vs. the primordial stable isotopes (atom-% abundance) given as range observed on earth.

$R = \text{isotope a} / \text{isotope b}$

Isotopic ratios of those elements are usually measured by Thermal Ionisation- (TI-MS)- or Inductively Coupled Plasma- (ICP-MS)-Mass Spectrometry  
According to: Horn et al., 1998

Element	symbol	mother nuclide	half-life time	R
strontium	<sup>87</sup> Sr <sup>86</sup> Sr (9.87 atom %)	<sup>87</sup> Rb	48.8 x 10 <sup>9</sup> years	0.702 - > 1
neodymium	<sup>143</sup> Nd <sup>144</sup> Nd (23.8 atom-%)	<sup>147</sup> Sm	106 x 10 <sup>9</sup> years	0.510–0.514
lead	<sup>208</sup> Pb <sup>204</sup> Pb (1.4 atom-%)	<sup>232</sup> Th	14.1 x 10 <sup>9</sup> years	33.0 – 52.0
lead	<sup>207</sup> Pb <sup>204</sup> Pb (1.4 atom-%)	<sup>235</sup> U	0.7038 x 10 <sup>9</sup> years	14.0 – 24.0
lead	<sup>206</sup> Pb <sup>204</sup> Pb (1.4 atom-%)	<sup>238</sup> U	4.468 x 10 <sup>9</sup> years	13.0 – 25.0

Table 3:

Officially acknowledged methods for food quality control based on stable isotope ratio analysis by isotope ratio mass spectrometry = IRMS or  $^2\text{H}$  nuclear magnetic resonance spectroscopy =  $^2\text{H}$ -NMR. 1 =  $^2\text{H}$ -NMR; 2 = year of official acknowledgement. S = sugar-; W = water-addition, CEN = European Commission for Normalization AOAC = Association of Official Analytical Chemists (USA)

Foodstuff	detection of	isotopic ratio	country/year2 institution
fruit juice	S	$^{13}\text{C}/^{12}\text{C}$ (sugars)	EU - CEN 1995
fruit juice	S	$^{13}\text{C}/^{12}\text{C}$ (sugars)	USA - AOAC 1981
fruit juice	S	$^{13}\text{C}/^{12}\text{C}$ (sugars and pulp)	EU - CEN 1998
fruit juice (concentrate)	S	$^{18}\text{O}/^{16}\text{O}$ (water)	USA - AOAC 1992
fruit juice	S	$^2\text{H}/^1\text{H}$ (ethanol)1	EU,USA;CEN/AOAC 1996
fruit juice	W	$^{18}\text{O}/^{16}\text{O}$ (water)	EU - CEN 1995
		$^2\text{H}/^1\text{H}$ (water)	
honey	S	$^{13}\text{C}/^{12}\text{C}$ (honey)	USA - AOAC 1978
honey	S	$^{13}\text{C}/^{12}\text{C}$ (honey and protein)	USA - AOAC 1991
wine	S	$^2\text{H}/^1\text{H}$ (ethanol)1	EU 1990
wine	W	$^{18}\text{O}/^{16}\text{O}$ (water)	EU 1997
wine	S	$^{13}\text{C}/^{12}\text{C}$ (ethanol)	EU 2003
maple sirup	S	$^2\text{H}/^1\text{H}$ and $^{13}\text{C}/^{12}\text{C}$ (ethanol)1	USA-AOAC 2001

The parameters as mentioned above cannot only be applied to control a wine for illegal additions of compounds, they can also be useful to check if a not adulterated wine complies with its labelled geographical origin or year of vintage, as there are climatic and environmental differences, eg mean temperature, amount of precipitation, air humidity, between different wine growing regions, or in one region between different vintages.

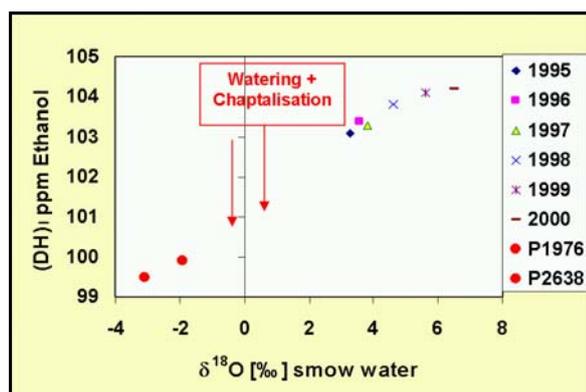


Figure 1: Examples for the seasonal variability of stable isotope data for authentic wines (mean values) and for adulterated (sugar and water addition) wines labelled to originate from the same region

In cases where the stable isotope data for commercial products are clearly outside the

total range for any authentic product (outside the so called cut-off values, e.g.  $\delta^{18}\text{O}$  value of wine water from Southern Europe with  $-2$  or  $-4$  ‰), it is not necessary to have a detailed database at all to prove the adulteration.

In other cases, and especially for the control of the labelled geographical origin and/or year of vintage this cannot be done without the database.

Unfortunately the EU wine database is until now only accessible for the laboratories contributing to the national wine databanks (the so-called member state labs = MSL), and even those laboratories have no access to the EU wine databank results from other countries. The only institution having all results available is until now the Joint Research Center of the EU (JRC) in Ispra (Italy), which has to be asked in cases where the data are required for food control, customs investigation, or court case requirements.

Within the last years, additional stable isotope parameters have been analysed and found applicable and useful for geographical origin assignment, not only of wine and fruit products, but even of animal food commodities as milk, butter, cheese, and meat (Bonner and Förstel 2004, Brescia et al., 2005, Camin et al. 2004, Camin et al. 2007, Crittenden et al. 2007, Manca et al. 2006, Pillonel et al. 2005, Rossmann et al. 2000, Rossmann 2001, Schmidt et al. 2005). Especially

for proteins, but even for plant materials the stable isotope ratios of elements nitrogen and sulphur have been used, and several heavy elements, preferably strontium have been found to be very useful for detection of the geographical provenance, too.

On the basis of the experiences with the EU wine databank, the Schutzgemeinschaft der Fruchtsaftindustrie e.V. (SGF e.V.) in 1991 started to build a database for fruit juice stable isotope results as well. This was done in the frame of an EU project from 1991 to 1993, and continued in a second EU project "PURE JUICE" from 2002-2005. This database is now accessible for public laboratories and EU control laboratories via internet as well ([www.purejuice.org](http://www.purejuice.org)), and it contains data for all light elements' stable isotope ratios and for strontium isotopic ratios for the commercially most important fruit juices (citrus, pineapple, apple) from the relevant provenance regions. With this interactive databank, own stable isotope results for a product can be plotted in the graphics build with the database values and can be compared in that with the results for all samples available in that database. As an example for its application, the strontium isotopic ratios for authentic orange juice (single strength juices) and commercial products labelled as single strength juices from a certain region are given (figure 2). It is obvious, that the authentic juices exhibit a close correlation of strontium isotope ratios for liquids and solids (pulp), while commercial products sometimes show remarkable deviations from that correlation, due to mixing of single strength juices from one provenance with rediluted concentrates from another region.

In 2005 the Arbeitsgruppe (AG) Stabilisotopenanalytik of the Gesellschaft Deutscher Chemiker e.V. (GDCh) decided to build their own database, starting with carbon and oxygen isotopic ratios of sugars and waters, respectively, from single strength apple juices of relevant

European provenance regions, which is accessible at [www.agstabilisotopen.org](http://www.agstabilisotopen.org) until now for the members of this group. An example for its application for differentiation of commercial single strength apple juice and rediluted apple juice from concentrate is given in figure 3.

Additional private or national databanks of stable isotope parameters exist eg for Grana Padano, parma ham, German and British and Austrian beef, honeys, German and Austrian asparagus, German fruit and grain spirits (Bauer-Christoph et al., 1997, Engel et al., 2007), Italian grape brandies. In general, the use of databases is necessary for stable isotope analyses, especially for the question of geographical origin assignment, but it would be nearly impossible to build up databases for all products which are or might be investigated by stable isotope methods. Furthermore, due to the global trade it must be possible to evaluate even products from very distant regions, from where authentic sample material is not available or only very difficult to obtain. This was one of the reasons for initiating the current EU project "TRACE" ([www.trace.eu.org](http://www.trace.eu.org)), which finally aims on the prediction of stable isotope data for products from a certain region on the basis of known isotopic data of precipitation, or climatic information, information about geography, geology and soil composition, and agricultural practises. This will be done by producing prediction models for products from European regions (Iceland to Greece, Ireland to Sicily, Spain to Poland) investigated with regard to their stable isotope content in TRACE, and being related to the relevant geological, climatic, hydrological, and geographical information, which includes the stable isotope data e.g. for ground waters, surface waters, soils and rocks. Supposed that such a system finally can be used for practical work, the same would be possible to be applied to products from other regions of the world, even if only climatic and geological /geographical information is accessible, but no authentic samples can be taken.

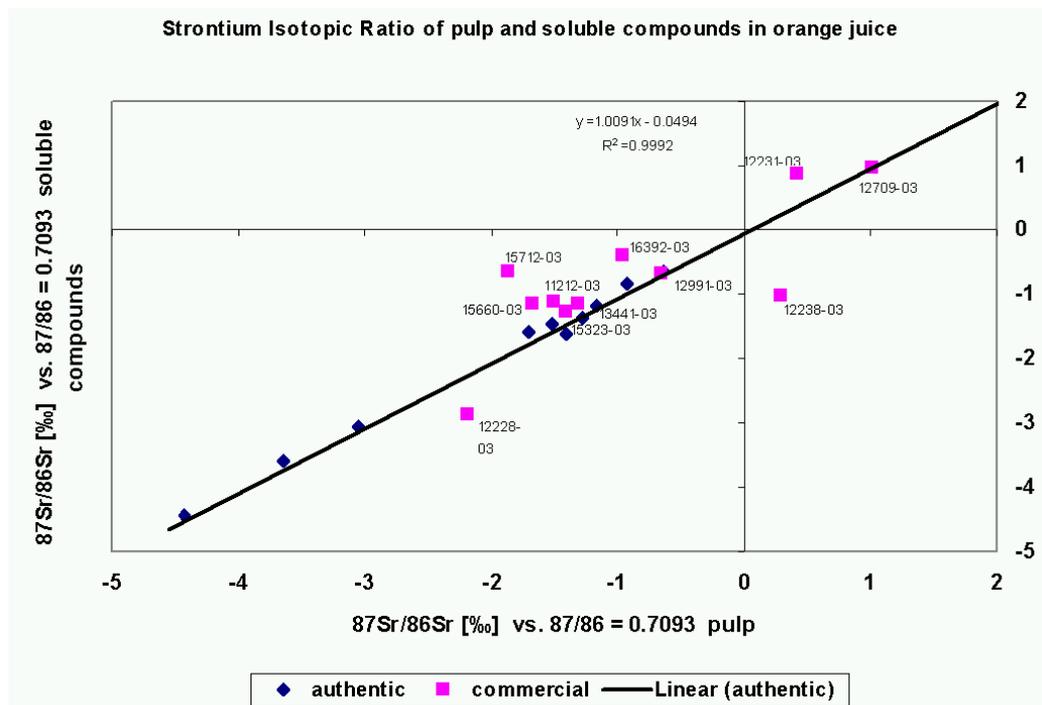


Figure 2:  
Strontium isotope correlation of solids (pulp) and liquids in authentic single strength orange juice and in commercial products labelled as single strength juices from the same provenance region

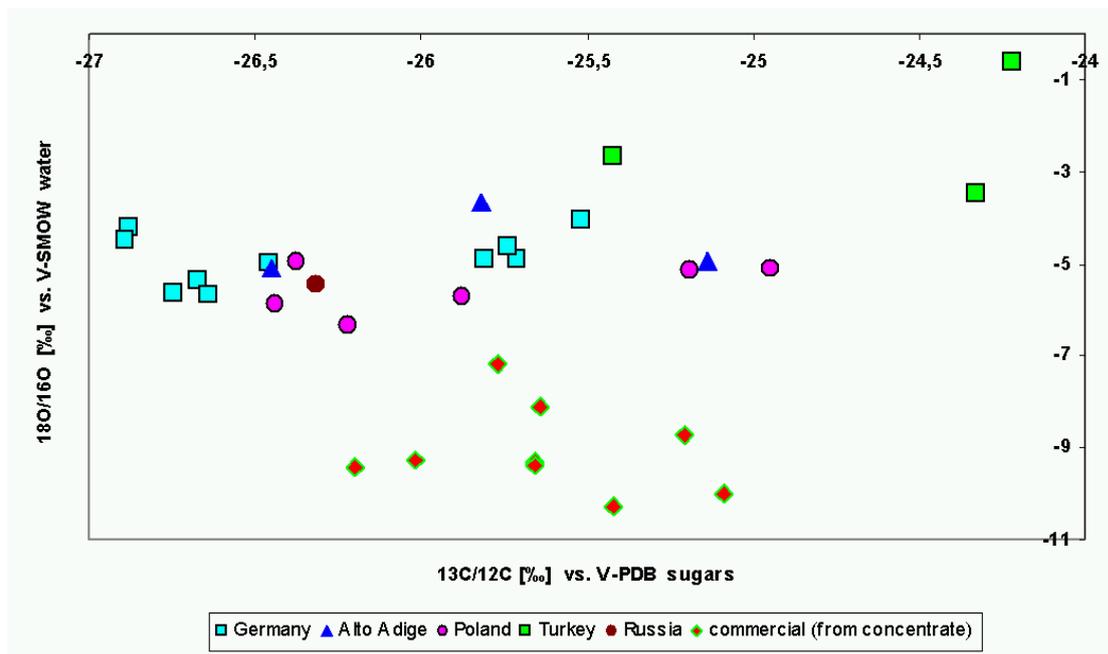


Figure 3:  
Carbon and oxygen isotopic ratios of sugars and water of authentic single strength apple juices and commercial apple juice made from redilution of apple juice concentrates

### 3 Stable isotope analyses for wood geographical provenance control

For the purpose of geographical origin assignment of wood, especially for wood from tropical regions, the experience and data as obtained for food products, namely for fruit juices, which come from tropical regions, too, can be very useful. As an example, the stable isotope characteristics for orange from Brazil or for pineapple, mango, and other tropical fruits from India or South-East Asia can indicate the expectation range for wood from the same regions very well. From experience with fruit juices, the combined application of hydrogen (or oxygen), carbon, sulphur, and strontium isotopic ratios, and the data evaluation using discriminant analyses is recommended (Camin et al., 2007). In the fruit juice business it is well-known, that most reliable results can be obtained by combining two or three different analytical methods, which would in case of wood support the use of genetic and

stable isotope analyses together for origin determination.

As the standard method for preparing a suitable probe from wood, the alpha-cellulose, which is applied for oxygen isotope and (after derivatisation) for hydrogen isotope analysis, is very laborious, recently another procedure has been proposed for the hydrogen isotope analysis of wood, aiming on relating the hydrogen isotope data of the organic compounds of wood to the precipitation water of the provenance region (Keppler et al., 2007). This method is based on the measurement of hydrogen isotopic ratios of lignin methoxyl groups as methyl iodine, which is easily prepared from dried and fine grind wood by addition of hydrogen iodine, and can be measured using GC-Pyrolysis-IRMS. As an example of the results obtained with that method, the correlation of the hydrogen isotopic data for methyl iodine prepared from wood and for water of the provenance regions are given in comparison with the hydrogen isotopic data for bulk wood from the same samples (figure 4).

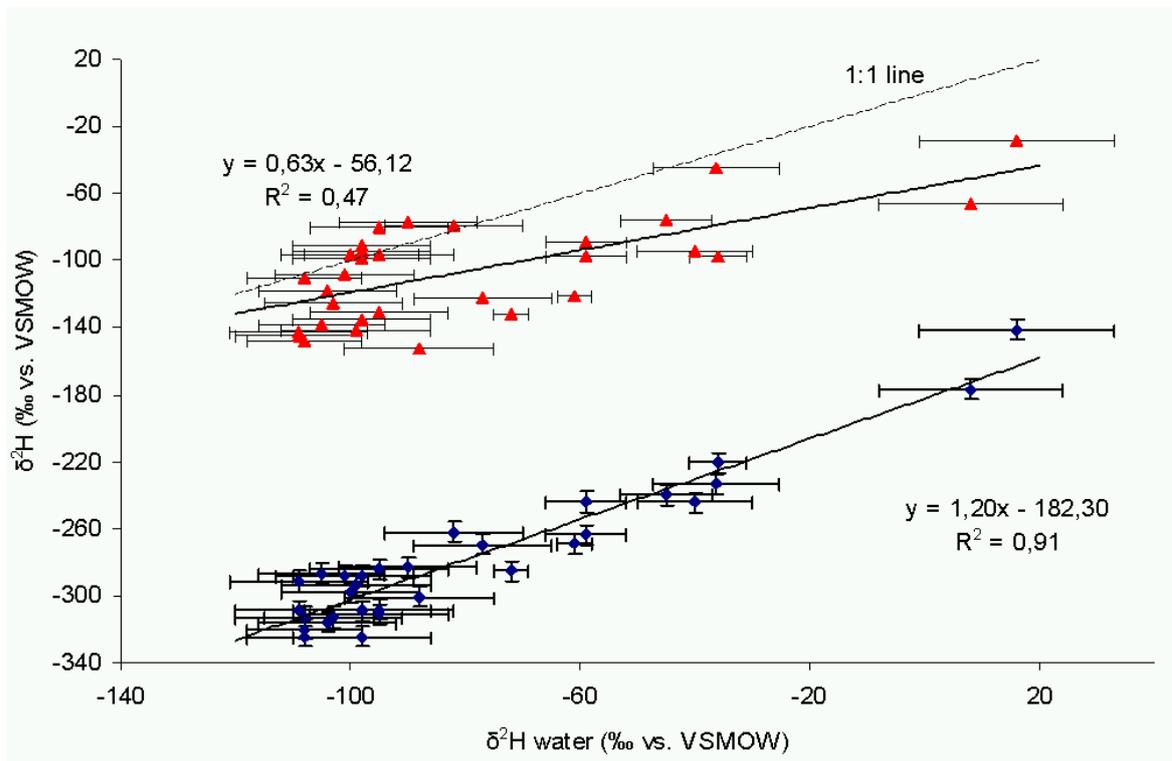


Figure 4: Hydrogen isotopic data for wood lignin methoxyl groups (blue marks) and bulk wood (red marks) relative to the hydrogen isotope data of meteoric water and respective correlation coefficient

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