Requirements for using multi element stable isotope analysis in food authentication

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Workshop: Determining the geographical origin of food
Fera, York, UK, February 28, 2014

Content

Elements: „bio“-elements, „geo“-elements

Isotopes, abundances, international standards

Instrumentation

Isotope ratio mass spectrometer, instrumental development

dual inlet IRMS ---> continuous flow IRMS

Methods

Official methods, methods to be developed/acknowledged

Reference materials and sample preparation

Official RM‘s, ICM‘s from TRACE, method testing, inter laboratory comparison

Databank and data evaluation

Types of analyses using stable isotopes for food control

“Traditional”

Authenticity check

is a given compound a natural product from a food material or has it been added ?

Natural or not natural

e.g. vanillin from beans or synthesized from lignin

“New”

Compliance with declaration

e.g. conventional or “organic” production

Geographical origin of (premium) products

e.g. PDO (Emmentaler) cheese or ham (Parma)

Elements and their stable isotopes, which are important as components of living matter (bioelements)

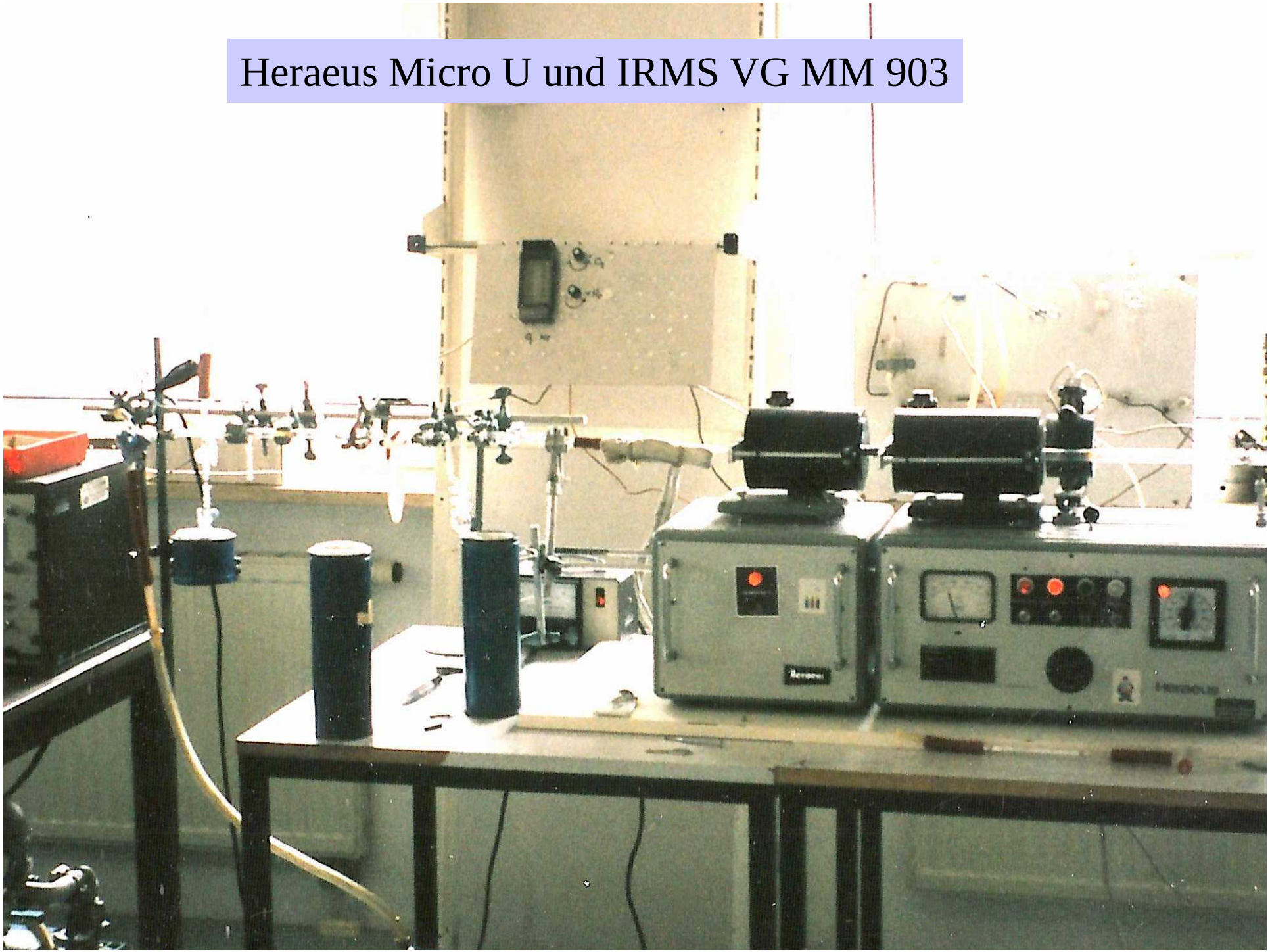
F = cIsotope/Sum cIsotope. R = cIsotope a/cIsotope b

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; Schmidt and Winkler, 1980)

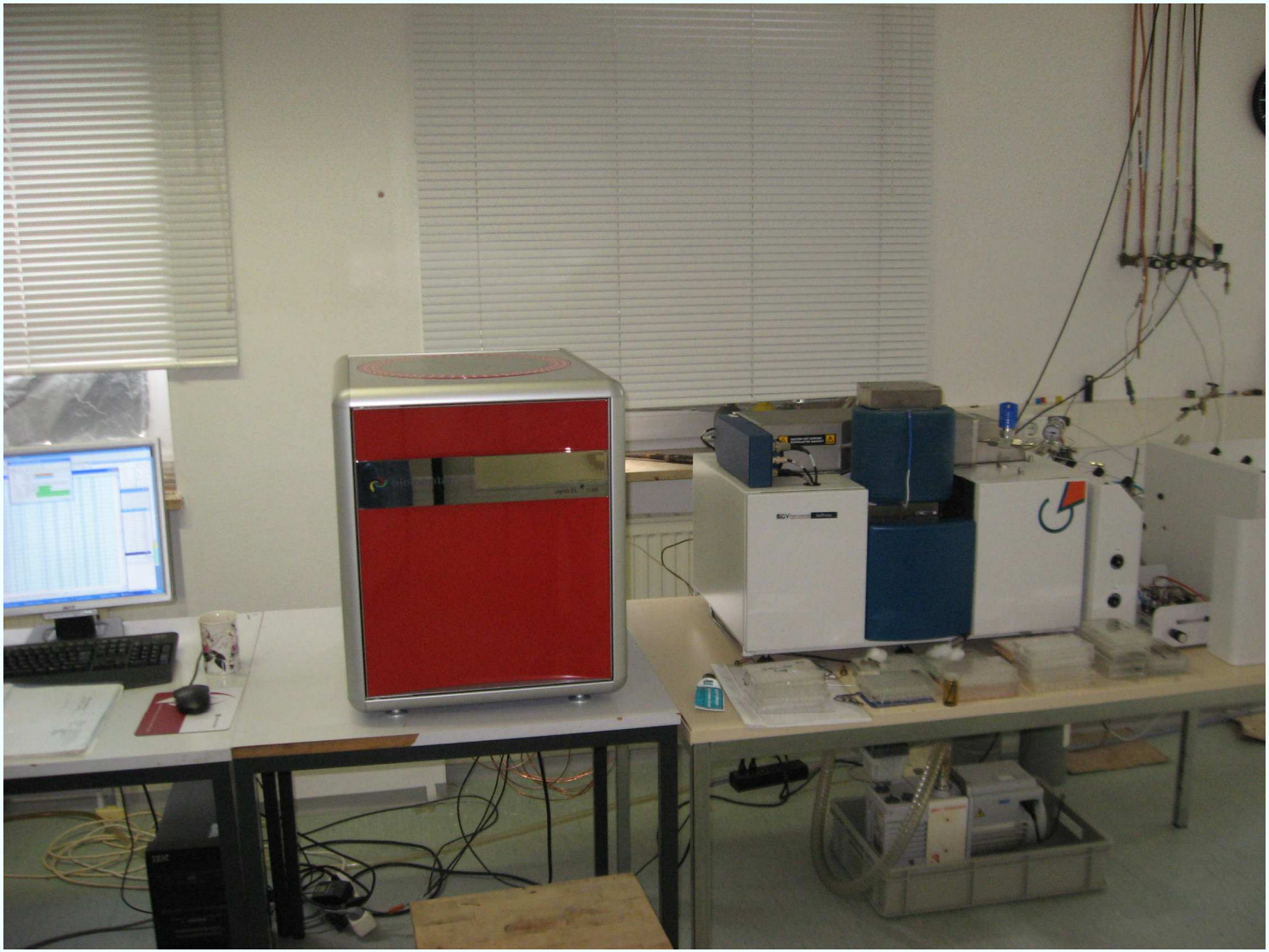
Element	symbol	Isotop F [atom-%]	standard name	R
hydrogen	^{1}H	99.9855	Standard Mean Ocean Water (=SMOW)	0.00015576
	$^{2}H=D$	0.0145		
carbon	^{12}C	98.892	Pee Dee Belemnite (=PDB)	0.011237
	^{13}C	1.108		
nitrogen	^{14}N	99.6337	Air (Air nitrogen) (=AIR)	0.0036765
	^{15}N	0.3663		
oxygen	^{16}O	99.7587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

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

Heraeus Micro U und IRMS VG MM 903



Simultaneous Analysis of C,N,H,S isotopic ratios: Vario Cube EL + Isoprime



Working time for sample preparation and analysis

sample preparation
casein preparation from cheese (cutting, drying, grinding, lipid extraction) 1 h per sample
methionin from casein (hydrolysis, derivatisation, preparative HPLC) 3 h per sample

off-line preparation of measuring gases

for hydrogen isotope analysis	**2 h per sample**
for carbon isotope analysis	**0,25 h per sample**
for nitrogen isotope analysis	**2 h per sample**
for oxygen isotope analysis	**2 h per sample**
for sulphur isotope analysis	**3 h per sample**

off-line measurement including calibration: **2,5 h per sample for 5 elements**

total working time casein isotopic analysis (H,C,N,S) ***13,25 h per sample***

total work time on-line measurement CNS	**1,5 h measurement + 1,25 h preparation + weighing**
total work time on-line measurement D/H	**0,5 h measurement + 0,25 h preparation + weighing**
sum for C,N,S + D/H of casein	**3,5 h per sample**

C,N,S + D/H of casein simultaneous ***2,75 h total working time***

Stable Isotope Reference Materials

- International accepted reference materials (IAEA)

  e.g. NIST-22 (oil), S-1 (AgS), N-1 (KNO_3), V-SMOW

  Available, but not suitable (no food components), not applicable for multi element analysis, elemental composition different from food

- Certified reference materials (CRM‘s, BCR sugars, ethanol)

  Not available for nitrogen and sulphur containing substances as proteins,

  USGS 42 and 43 hair reference materials not well suitable (too high sulfur content)

- Interlaboratory intercomparison materials (ICM‘s)

  Casein, collagen, meat proteins, wheat flour

  Selected for TRACE, but not officially acknowledged

- Intralaboratory working standards (casein, collagen,hair, wheat flour)

- Development of suitable standards will proceed with increasing demand

Method testing, official methods and proficiency tests

- **Official methods** since 1978 in US and EU,
  for honey, fruit juices, wine, maple syrup (table)

- Methods developed are tested in **round robin experiments**
  (sample preparation and isotopic analysis)

- Ability of laboratories to give correct data is controlled
  by **proficiency tests** using the official methods
  (Typical samples, eg wines are circulated and analysed
  3 times per year)

Officially acknowledged methods for food quality control based on stable isotope ratio analysis by isotope ratio mass spectrometry = IRMS or ^{2}H nuclear magnetic resonance spectroscopy = ^{2}H-NMR

1 = ^{2}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}C/^{12}C (sugars)	EU - CEN 1995
fruit juice	S	^{13}C/^{12}C (sugars)	USA - AOAC 1981
fruit juice	S	^{13}C/^{12}C (sugars and pulp)	EU - CEN 1998
fruit juice (concentrate)	S	^{18}O/^{16}O (water)	USA - AOAC 1992
fruit juice	S	^{2}H/^{1}H (ethanol)1	EU,USA;CEN/AOAC 1996
fruit juice	W	^{18}O/^{16}O (water) ^{2}H/^{1}H (water)	EU - CEN 1995
honey	S	^{13}C/^{12}C (honey)	USA - AOAC 1978
honey	S	^{13}C/^{12}C (honey and protein)	USA - AOAC 1991
wine	S	^{2}H/^{1}H (ethanol)1	EU 1991
wine	W	^{18}O/^{16}O (water)	EU 1996
wine	S	^{13}C/^{12}C (ethanol)	EU 2003
maple sirup	S	^{2}H/^{1}H and ^{13}C/^{12}C (ethanol)1	USA-AOAC 2001
cheese	origin	C (N,H,S)	EU Reg 584/2011

In-house validation of preparation and isotopic analysis of animal protein

Sample drying, lipid extraction, simultaneous CNS isotope analysis,
H isotope analysis using high temperature conversion

	$\delta^{15}N$		$\delta^{13}C$		$\delta^{34}S$		$\delta^{2}H$	
		Sd		sd		sd		sd
Huhn 09-06-1370 I	**3,04**	**0,02**	**-20,95**	**0,04**	**6,65**	**0,06**	**-106,28**	**0,80**
Huhn 09-06-1370 II	**3,1**	**0,02**	**-21,07**	**0,07**	**6,78**	**0,05**	**-106,24**	**0,33**
Huhn 09-06-1370 III	**3,05**	**0,01**	**-20,96**	**0,06**	**6,63**	**0,05**	**-108,17**	**0,55**
Huhn 09-06-1370 IV	**3,04**	**0,02**	**-21**	**0,06**	**6,7**	**0,03**	**-106,60**	**0,54**
Huhn 09-06-1370 V	**3,03**	**0,13**	**-21**	**0,1**	**6,78**	**0,12**	**-107,15**	**0,33**
Total mean	**3,05**		**-21,00**		**6,71**		**-106,89**	
sd	**0,02**		**0,04**		**0,06**		**0,72**	

Especially important hydrogen isotope analysis of proteins:
Comparative equilibration technique
(Wassenar and Hobson, 2003) – see below.

Inter-laboratory reproducibility for the freeze dried lamb ICM (lamb ICM 050818) (SR = standard deviation of reproducibility)

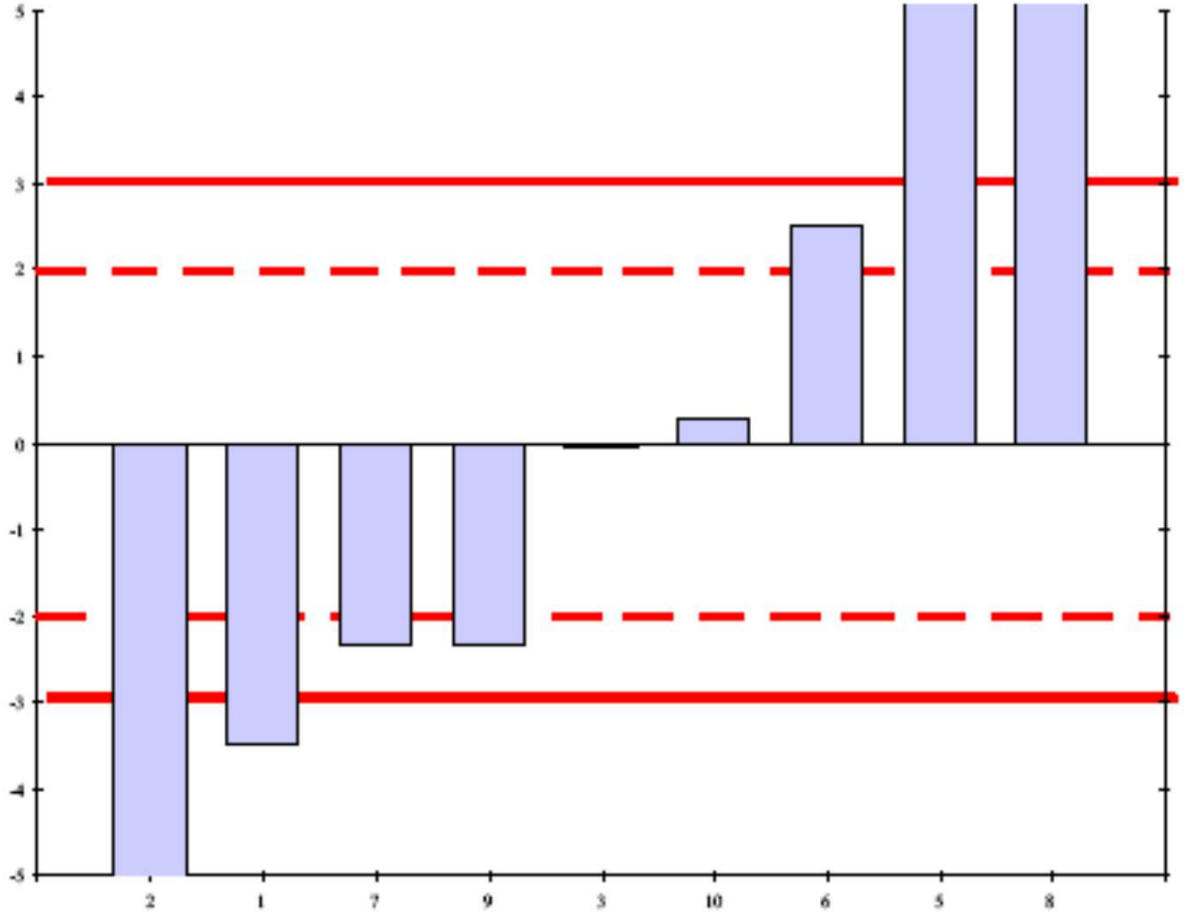
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	mean	SR
$\delta^{13}C$ [‰]$_{V-PDB}$	-26.62	-26.90	-26.65	-27.01	-26.83	-26.62	**-26.66**	**0.16**
$\delta^{15}N$ [‰]$_{AIR}$	7.56	7.51	7.95	7.67	7.50	7.52	**7.53**	**0.2**
$\delta^{2}H$ [‰]$_{V-SMOW}$	-102.6	-96.4	-96.2	-95.9	-98.4	-94.3	**-97.7**	**4.4**
$\delta^{34}S$ [‰]$_{V-CDT}$	5.16					5.30	**5.28**	**0.2**

Good agreement of hydrogen isotopic data for proteins between laboratories using comparative equilibration measurement !

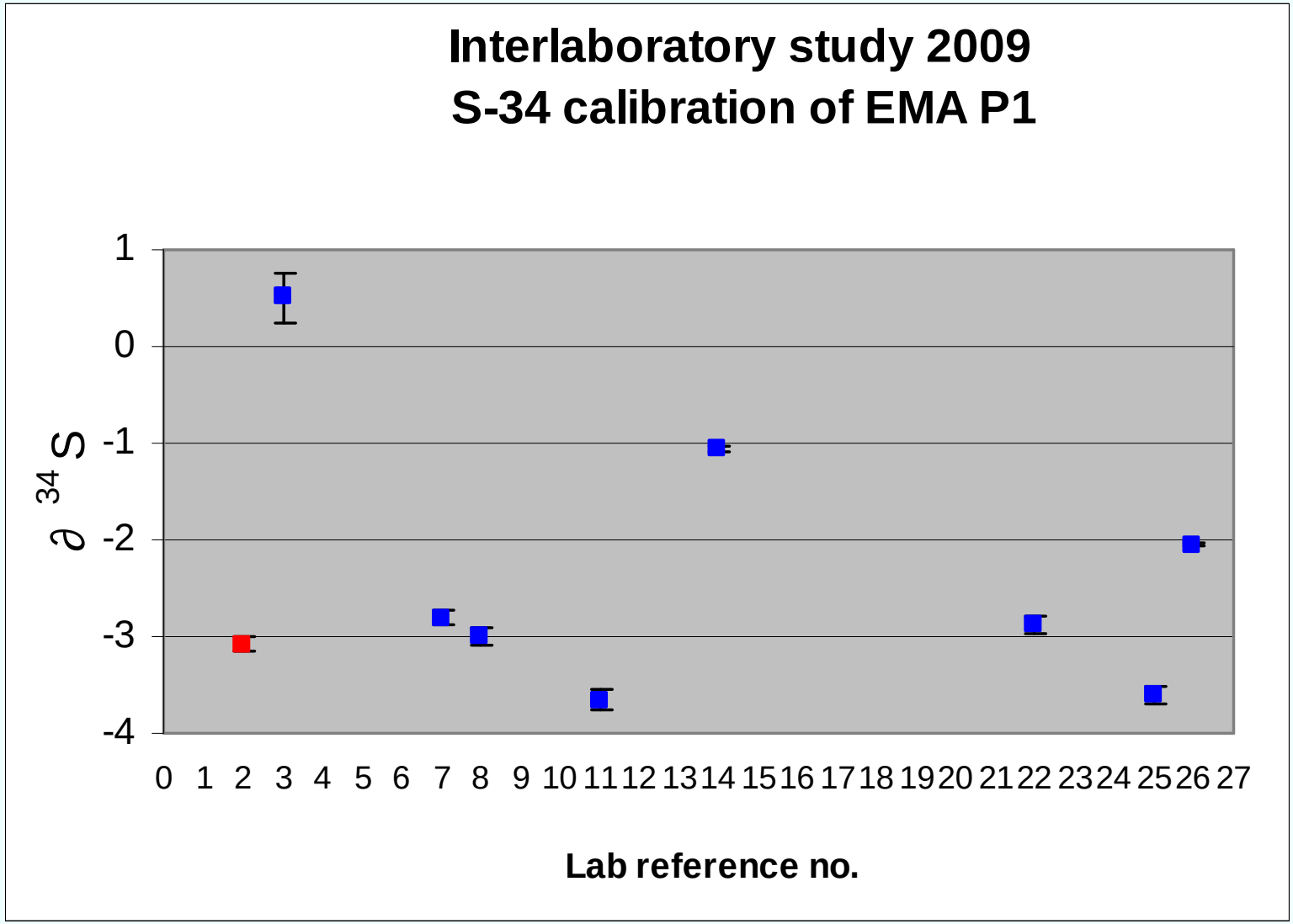
Proficiency Test $^{15}N/^{14}N$ in wheat flour

Round	Product:	Parameter:
2009 Round 3	Flour	N15

Laboratory Code	N15	Z-scores
2	1.79	-5.90
1	2.15	-3.90
7	2.36	-2.33
9	2.36	-2.33
3	2.77	-0.05
10	2.83	0.28
6	3.23	2.50
5	3.82	5.78
8	5.67	16.06



Inter laboratory Test



Inter laboratory Test

RESULTS

PTS Jue / Okt_2013

Peanuts	JUE/1013/2

	$^{18}O/^{16}O$ [‰] v.s. vsmow		D/H [‰] v.s. vsmow		13C/^{12}C [‰] v.s. PDB		15N/^{14}N [‰] v.s. Air		34S/^{32}S [‰] v.s. CDT	
Nummer	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
01	14,9	0,8	-94,6	0,9	-25,0	0,1	-0,4	0,3	5,3	0,4
02					-25,1	0,1	-0,3	0,1		
03	14,1	0,2	-92,4	1,4	-25,1	0,0	-0,4	0,1	4,1	0,3
04	16,3	0,4	-100,1	3,0	-25,0	0,2	-0,2	0,2		
05			-100,3	3,0	-24,9	0,1	-0,6	0,2	4,5	0,3
06					-24,8	0,2	-0,3	0,2		

n	3	4	6	6	3
mean	15,1	-96,9	-25,0	-0,4	4,6
median	14,9	-97,4	-25,0	-0,4	4,5
SD	1,1	4,0	0,1	0,1	0,6

Collection of data for authentic samples (databanks)

- European wine databank (since 1990, JRC Ispra)

- National databanks for wine, spirits, fruit juices, asparagus, cheeses, honey, (eg data collection of German public control laboratories)

- Industrial databanks (e.g. fruit juice) – AIJN code of practise

- Apple juice data bank of AG „Stabile Isotope“ of GDCh

- European databanks from EU projects (milk and cheese, fruit juice, data from project TRACE)

General problem: access to data collections

Data evaluation methodology

Comparison of isotopic data (delta values) with "cutoff-values" – an authentic/natural product cannot have the measured value

Method of internal standard – isotopic result for a questionable compound is compared with the result for another compound which is usually not added e.g. bulk honey and honey protein $\delta^{13}C$ value

For geographical origin determination usually this cannot be applied, as this requires determination of

Multi element stable isotope pattern

e.g. hydrogen, carbon, nitrogen, sulphur isotope data combined with sophisticated data evaluation methods as discriminant analysis
Sometimes "geo-elements" as strontium, boron can be useful

Anyway, a database of authentic samples is required
There should be general rules for data interpretation

DATA PREDICTION

- Prediction models should reduce the need of databanks

  They should enable to estimate stable isotope data for food products from regions were no samples had been analysed so far

- Based on TRACE experience, it is possible to predict stable isotope pattern of elements HCNS, but not exact values
- Even if climate and continentality are very important, they cannot explain the total variability of stable isotope data (seasonal effects, geology, soil conditions, agricultural practises, microclimate have to be considered as well)
- At present, models cannot replace databanks, but justify reduction in number of databank samples

Conclusion

- Stable Isotope Analysis is an efficient tool for authenticity control of foodstuff (proof of adulteration and detection of mislabelling of geographical origin)

- ICM materials were selected and preparation methods have been developed and tested successfully. Official certified reference materials are not available. Intralaboratory and interlaboratory reproducibility and agreement are satisfactory.

- Reliable assignment especially with regard to geographical origin usually requires multielement stable isotope analyses (H,C,N,O,S and eg Sr,B) and appropriate data evaluation methods.

- the aim of prediction of stable isotope data for products from certain regions based on models using climatic, geographical and geological data (maps) can be achieved qualitatively, but not quantitatively (no prediction of certain numbers)