Upcoming food matrix stable isotope reference materials from the USGS:
honeys, vegetable oils, flours, and collagens

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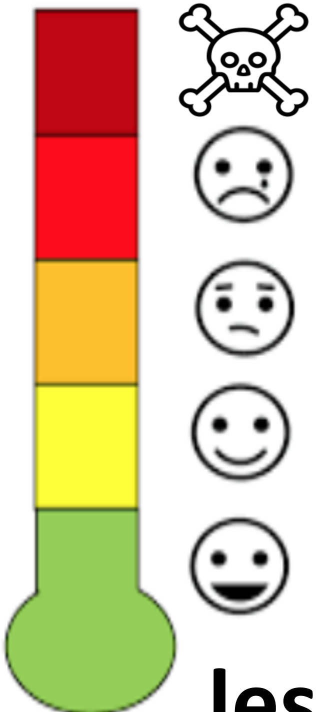
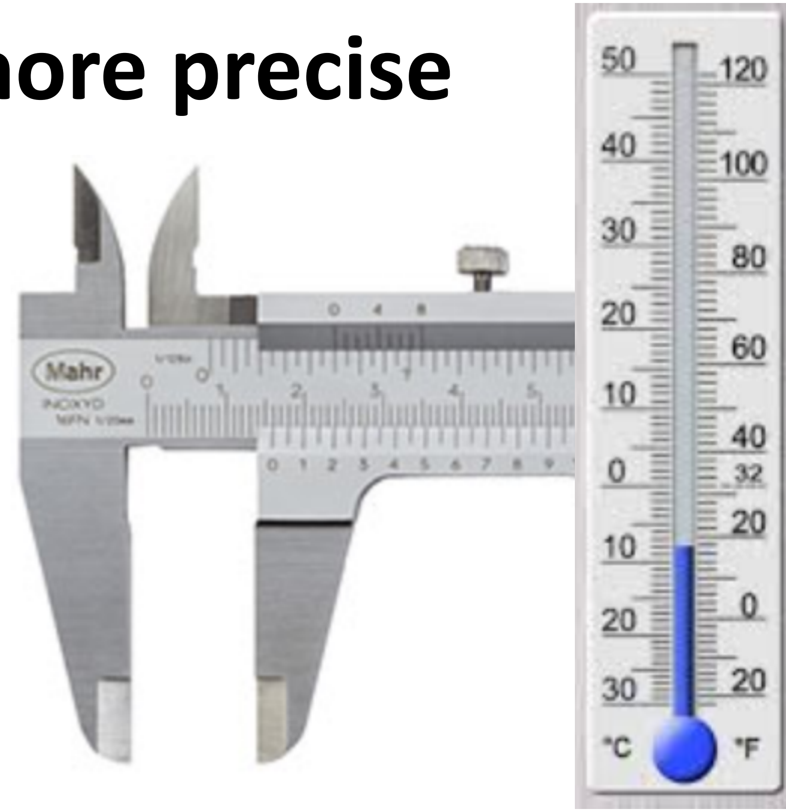


less precise

more precise



Reference Materials (RMs) are needed to position your data along conventional δ-scales.

Internationally accepted reference materials

Primary RMs

- define the position of the zero-point. These materials have exact isotope δ-values by consensus with no uncertainty. Over time some primary RMs are known to have developed problems.

Secondary RMs

- are natural or synthetic compounds that have been carefully calibrated relative to the primary RMs.The δ-values of secondary RMs are subject to amendments and always carry analytical uncertainty. It is recommended that authors publish the used δ-values.

Lower-level RMs

- can be calibrated against higher-level RMs by laboratories to serve as working RMs.

RMs are not permanent!

Table 2 The δ^2H values of hydrogen isotopic reference materials.[a]

Description	NIST RM #	Material	δ^2H_{VSMOW}	Uncertainty	References	Comment
VSMOW	8535	Water	**0**[b]	None	[34]	Quarantined[c]
SLAP	8537	Water	−428.5 ‰	0.1	[35]	Quarantined[c]
			−427.8 ‰	0.5	[36]	
			−428.8 ‰	1.3	[37]	
			−425.8 ‰	1.0	[38]	
			−428 ‰[b]	None	[34]	
SMOW		n/a	0	–	[39]	Scale discontinued [4]
VSMOW2		Water	**0**	0.3 ‰	[40]	
SLAP2		Water	**−427.5 ‰**	0.3 ‰	[40]	
GISP	8536	Water	**−189.7 ‰**	0.9 ‰	[41]	
GISP2		Water	−258.3 ‰	0.3 ‰	IAEA	Not yet released
NBS 1		Water	**−47.6 ‰**	n/a	[39]	Exhausted
NBS 1a		Water	**−183.3 ‰**	n/a	[39]	Exhausted
USGS45		Water	−10.3 ‰	0.4 ‰	[42]	
USGS46		Water	−235.8 ‰	0.7 ‰	[43]	
USGS47		Water	−150.2 ‰	0.5 ‰	[44]	
USGS48		Water	−2.0 ‰	0.4 ‰	[45]	
NBS 22	8539	Oil	−119.2 ‰	0.7 ‰	[46]	
			−116.9 ‰	0.8 ‰	[47]	
NBS 30	8538	Biotite	−65.7 ‰	0.3 ‰	[41]	
NGS1	8559	Natural gas (coal origin)	−138 ‰ (CH_4)	~5 ‰	[48]	Exhausted
NGS2	8560	Natural gas (petroleum origin)	−173 ‰ (CH_4)	~2.5 ‰	[48]	Exhausted
NGS2	8560	Natural gas (petroleum origin)	−121 ‰ (C_2H_6)	~7 ‰	[48]	Exhausted
NGS3	8561	Natural gas (biogenic)	−176 ‰ (CH_4)	~1 ‰	[48]	Exhausted
IAEA-CH-7	8540	Polyethylene foil	**−100.3 ‰**	2.0 ‰	[41]	
USGS42		Human hair (Tibetan)	**−78.5 ‰**	2.3 ‰	[49, 50]	
USGS43		Human hair (Indian)	**−50.3 ‰**	2.8 ‰	[49, 50]	

[a]Values for hydrogen isotope deltas are supplied with one place after the decimal point. They are listed in chronological order of the cited literature. In the case of multiple entries, values recommended by the Commission of Isotopic Abundances and Atomic Weights (CIAAW) are listed in bold font; those defining a scale are underlined in bold font. The latter have no associated uncertainty (by definition).

[b]Exact values defining the $\delta^2H_{VSMOW\text{-}SLAP}$ scale. Please note that both scale-defining materials, VSMOW for the scale origin and SLAP for the scale span, are given without uncertainty. These are fixed consensus values. They cannot be changed without changing the scale as well.

[c]Still available from the Reston Stable Isotope Laboratory of the U.S. Geological Survey.

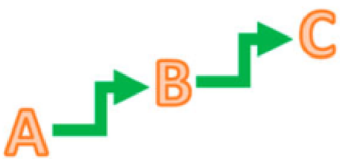
Primary RMs

Secondary RMs

Some of the values has been updated – see Coplen & Qi, FSI, 2016

Brand *et al*. (2014) https://doi.org/10.1515/pac-2013-1023

Planning of analyses



Obtain ≥ 2 suitable international primary or secondary isotope reference materials (RMs) for your analytical application that should **bracket the δ-values that you expect** from your unknown samples.

Optional: At a time when your stable isotope ratio analytical instruments work well, calibrate your own **tertiary laboratory RMs** against international RMs. The chosen RMs should bracket the δ-values that you expect from your unknown samples.

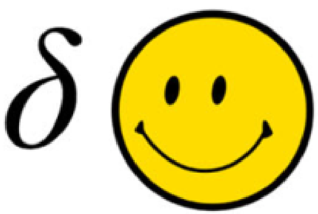
Performing analyses

Measure the ≥ 2 RMs and your unknown samples in a single analytical session in identical fashion, thus adhering to the **principle of Identical Treatment (IT) of standard and sample**. The resulting δ-values are raw, uncalibrated data.

Scale normalisation

Use proper statistical methods to **linearly scale-normalise raw δ-values** towards matching of δ-values of RMs with their prescribed δ-values.

Reporting of data



When describing analytical methods, explain which primary, secondary or tertiary RMs were used and **which δ-values were assigned to each RM**.

Propagate the errors of scale-normalised δ-values based on the empirically determined precision of repeat analyses and the accuracy of RM δ-values.

Use **SI-mandated and IUPAC-recommended nomenclature** when naming isotopes and expressing δ-values in terms of permil (‰) or milliurey (mUr).

Flowchart for isotope abundance analysis observing the identical treatment principle for both samples and RMs serving as scale anchors.

Meier-Augenstein and Schimmelmann (2019) *Isotopes in Environmental and Health Studies* **55** (2), 113-128.
https://doi.org/10.1080/10256016.2018.1538137

The principle of Identical Treatment (IT) of sample and reference material

- Ideally the **chemical nature of the sample should be identical to that of the reference material**. For example, use honey RMs when measuring and calibrating honey samples.

- Gases like H_2, CO_2, CO, N_2 and SO_2 from cylinders can only serve as **monitoring gases** in on-line applications because they have not passed through the same analytical sequence as analyte gases. **DO NOT CALL THEM REFERENCE GASES!**

Werner RA, Brand WA (2001) Referencing strategies and techniques in stable isotope ratio analysis. Rapid Communications in Mass Spectrometry 15, 501−519. http://dx.doi.org/10.1002/rcm.258

Upcoming USGS food matrix reference materials

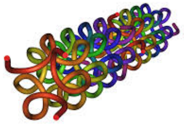
Honeys (carbohydrate-rich):

- honey from tropical southern Vietnam (collected fresh from wild bee hives)
- honey from prairie near Saskatoon, Canada (commercial product)

Vegetable oils (lipids):

- olive oil from Sicily, Italy (blended oil provided by Federica Camin)
- olive oil from coastal desert in Peru (commercial product)
- peanut oil from southern Vietnam (directly from rural producer)
- corn oil (i.e. from a C4 plant, ^{13}C-rich; commercial product from U.S.A.)

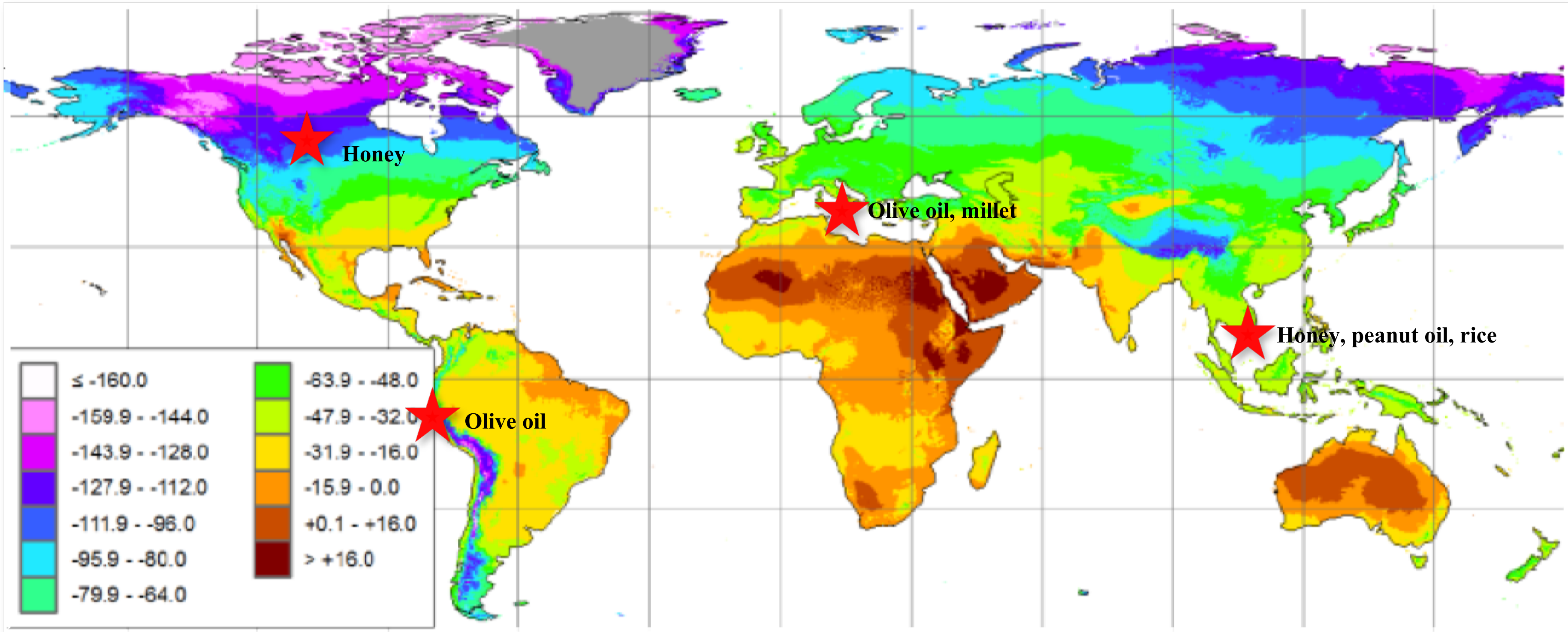
Collagens (proteinaceous):



- marine collagen powder from wild-caught fish (commercial product)
- porcine collagen powder (powder, provided *via* FAO/IAEA)

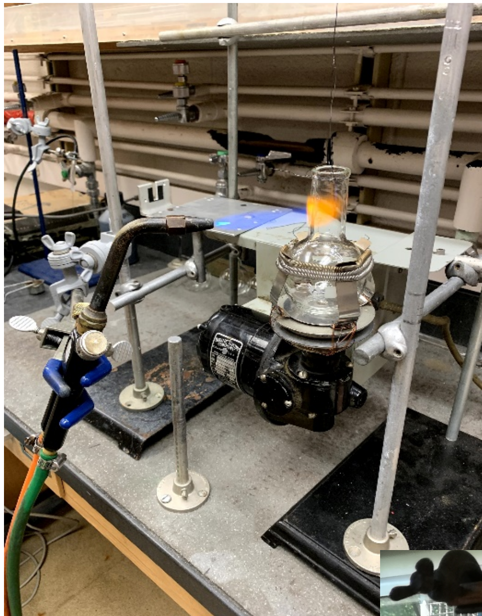
Flours (carbohydrate-rich):

- flour from millet (provided by Federica Camin, from Tuscany in Italy)
- flour from rice, from southern Vietnam (directly from local producer)



Predicted amount-weighted mean growing-season δ^2H (‰) in precipitation (Terzer et al., 2013, Hydrology and Earth System Sciences, 17, 1–16. https://doi.org/10.5194/hess-17-4713-2013).

Processing of oil



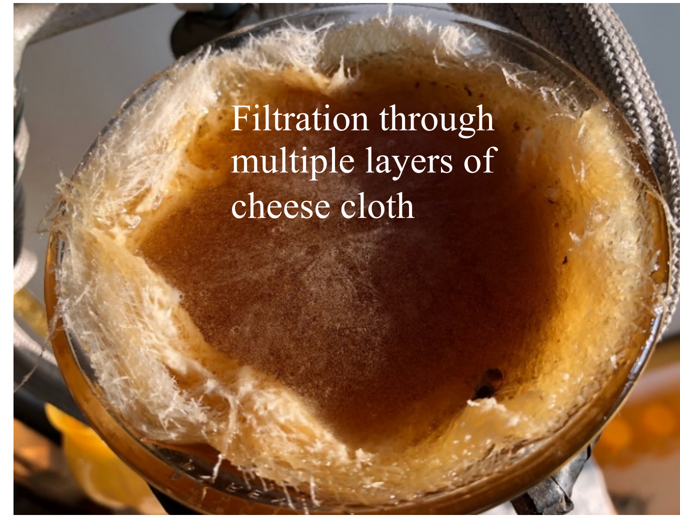
Heating neck of round-bottom borosilicate flask

Glass flasks ready for sealing under vacuum

Processing of honey


Fresh raw honey in southern Vietnam

Filtration of honey


Filtration through multiple layers of cheese cloth


Heated tent for dripping honey into glass flasks

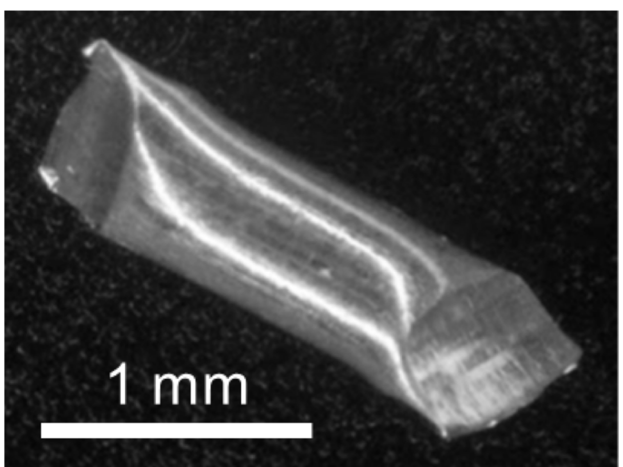

Filtered honey


Honey sealed in glass under vacuum


Filling honey into glass ampoules

Our final RMs after labelling, sealing under vacuum, and packaging in Ljubljana


The absence of leaks was verified by sparking with Tesla coil.



Liquid samples (waters, vegetable oils and honeys) can be crimp-sealed into segments of silver tubing for loading into EA carousels. Liquid food matrix RMs are available from the USGS, Reston, Virginia, USA.

Five participating ring-test laboratories for measuring bulk values

Laboratory	H	C	N	O	S	$H,O_{exchange}$
Fondazione Edmund Mach, San Michele all'Adige, Italy (Federica Camin)	+	+	+	+	+	+
Food and Environmental Protection Laboratory, IAEA, Seibersdorf, Austria (Simon Kelly)	-	+	+	-	+	-
Forensic and Scientific Services, Queensland, Australia (Jim Carter)	+	+	+	+	-	-
Jožef Stefan Institute, Ljubljana, Slovenia (Nives Ogrinc)	-	+	+	-	+	-
USGS, Reston, Virginia, USA (Ty Coplen, Haiping Qi)	+	+	+	+	+	+

Statistical evaluation:

- National Measurement Laboratory, LCG Ltd., Teddington, United Kingdom (P. Dunn)

Primary and secondary international standards and RMs used in ring test for calibration and normalisation of stable isotope ratio along conventional delta scales

Reference material	***Source of values***	***Accepted isotope values in mUr with uncertainties[&]***				
		δ^2H_{VSMOW}	$\delta^{13}C_{VPDB}$	$\delta^{15}N_{AIR}$	$\delta^{18}O_{VSMOW}$	$\delta^{34}S_{VCDT}$
IAEA-600 caffeine	Schimmelmann et al. (2016)	-156.1 ± 1.3	-27.73 ± 0.04	+1.02 ± 0.05	n.d.	n.a.
IAEA-601 benzoic acid	Brand et al. (2014)	n.d.	-28.81 ± 0.04	n.a.	+23.14 ± 0.19	n.a.
IAEA-N-1 ammonium sulfate	Brand et al. (2014)	n.d.	n.a.	+0.43 ± 0.07	n.d.	n.d.
IAEA-N-2 ammonium sulfate	IAEA (1995)*	n.d.	n.a.	+20.41 ± 0.12*	n.d.	n.d.
IAEA-S-2 silver sulfide	Mann et al. (2009)	n.a.	n.a.	n.a.	n.a.	+22.62 ± 0.16
IAEA-S-3 silver sulfide	Mann et al. (2009)	n.a.	n.a.	n.a.	n.a.	-32.49 ± 0.16
NBS 19 calcium carbonate	Brand et al. (2014)	n.a.	+1.95	n.a.	+28.65	n.a.
NBS 22a vacuum oil	Schimmelmann et al. (2016)	-120.4 ± 1.0	-29.72 ± 0.04	n.a.	n.d.	n.a.
NBS 127 barium sulfate	Paris et al. (2013)	n.a.	n.a.	n.a.	+8.59 ± 0.26	+21.12 ± 0.22
SLAP2 water	Brand et al. (2014)	-427.5 ± 0.3	n.a.	n.a.	-55.5 ± 0.02	n.a.
UCO4 water	USGS website**	+113.6 ± 0.5	n.a.	n.a.	+38.95 ± 0.04	n.a.
USGS44 calcium carbonate	USGS website**	n.a.	-42.10 ± 0.05[#]	n.a.	n.d.	n.a.
USGS61 caffeine	Schimmelmann et al. (2016)	+96.9 ± 0.9	-35.05 ± 0.04	-2.87 ± 0.04	n.d.	n.a.
USGS62 caffeine	Schimmelmann et al. (2016)	-156.1 ± 2.1	-14.79 ± 0.04	+20.17 ± 0.06	n.d.	n.a.
USGS64 glycine	Schimmelmann et al. (2016)	n.d.	-40.81 ± 0.04	+1.76 ± 0.06	n.d.	n.a.
USGS65 glycine	Schimmelmann et al. (2016)	n.d.	-20.29 ± 0.04	+20.68 ± 0.06	n.d.	n.a.
USGS66 glycine	Schimmelmann et al. (2016)	n.d.	-0.67 ± 0.04	+40.83 ± 0.06	n.d.	n.a.
USGS77 polyethylene powder	Schimmelmann et al. (2016)	-75.9 ± 0.6	-30.71 ± 0.04	n.a.	n.a.	n.a.
VSMOW water	Brand et al. (2014)	0	n.a.	n.a.	0	n.a.
VSMOW2 water	Brand et al. (2014)	0 ± 0.3	n.a.	n.a.	0 ± 0.02	n.a.

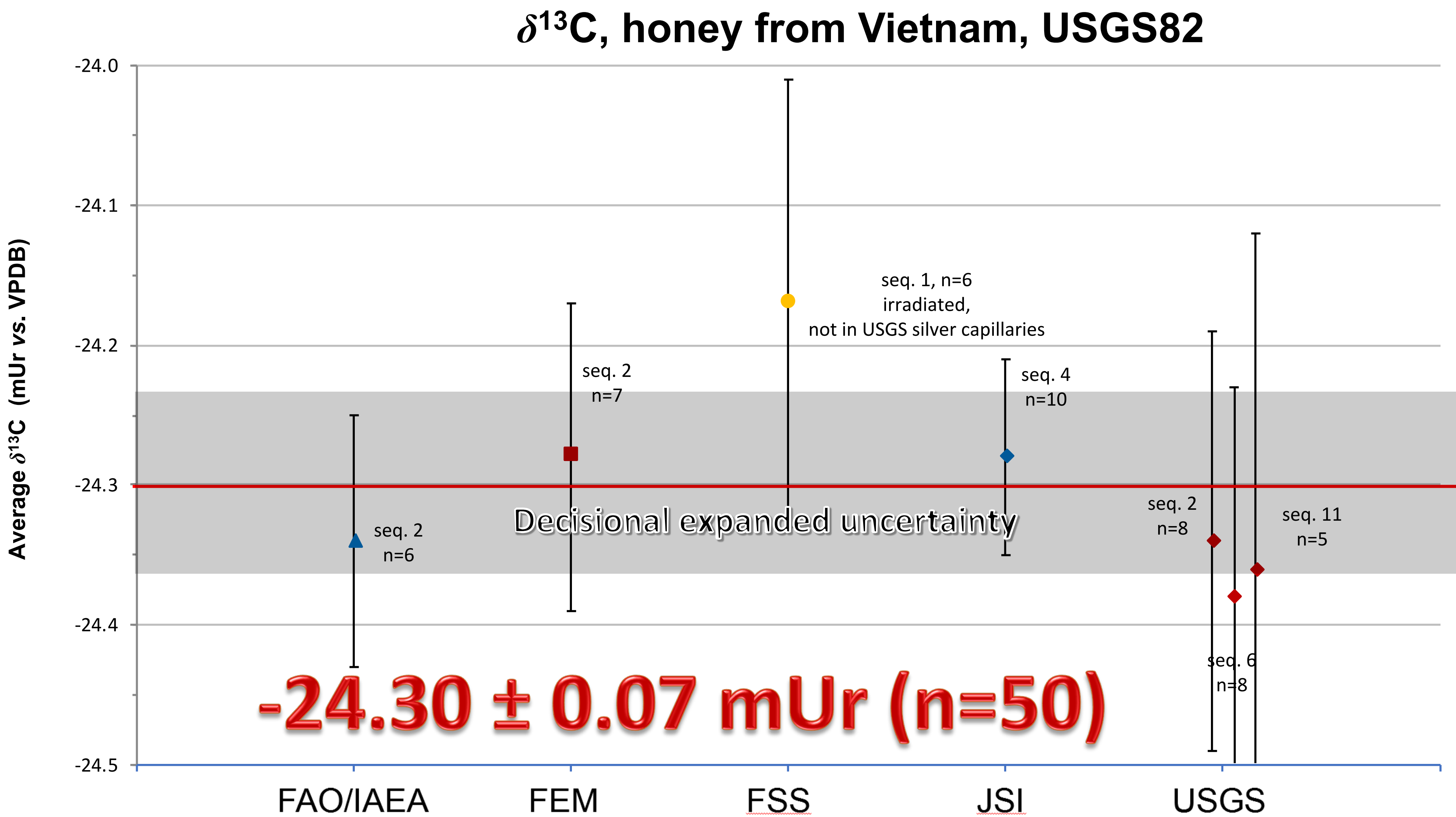
n.a. = not applicable; n.d. = not determined

* IAEA (1995) TECDOC-825, page 57. The value was normalized by assigning IAEA-N-1 to +0.43 mUr and USGS32 to +180 mUr.

** https://isotopes.usgs.gov/lab/referencematerials.html

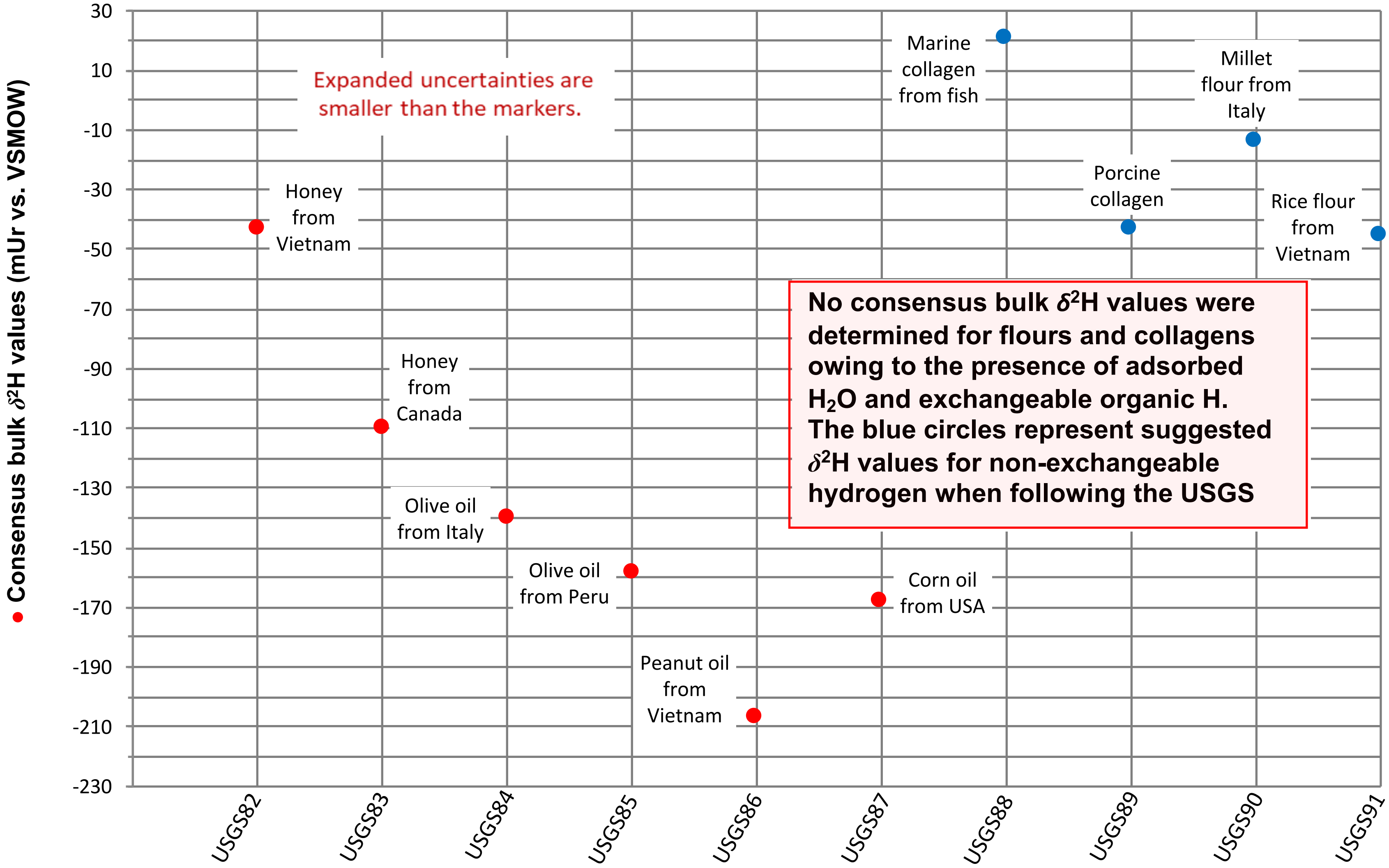
[#] value independent of LSVEC as determined by W.A. Brand; the value would be ~ -42.21 mUr if calibrated with LSVEC.

[&] uncertainties as indicated in sources of values. Further details about uncertainties offered in Supplemental Information.

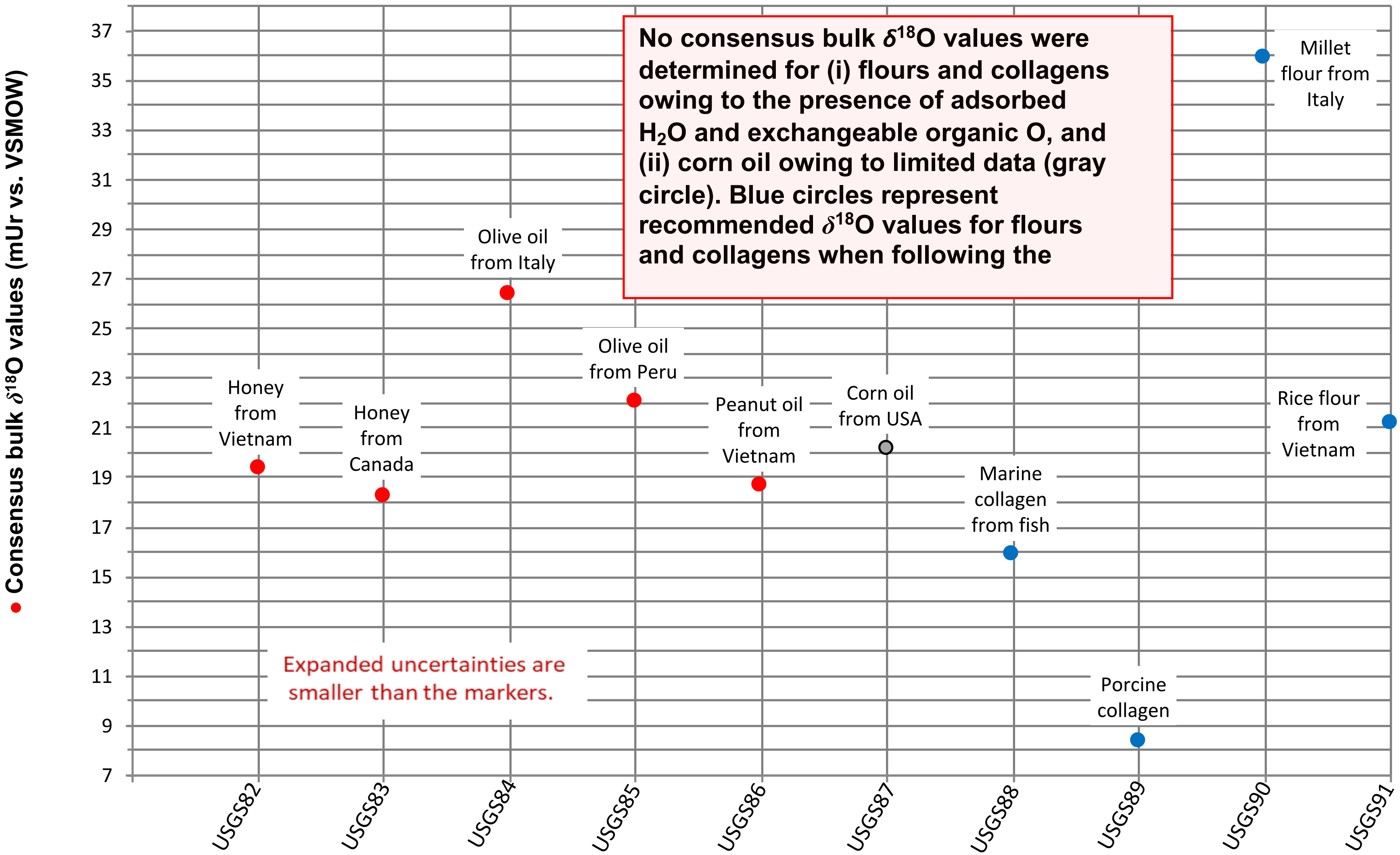


δ13C, honey from Vietnam, USGS82
Average δ13C (mUr vs. VPDB)
-24.0
-24.1
-24.2
-24.3
-24.4
-24.5
seq. 1, n=6
irradiated,
not in USGS silver capillaries
seq. 2
n=7
seq. 4
n=10
seq. 2
n=6
Decisional expanded uncertainty
seq. 2
n=8
seq. 11
n=5
seq. 6
n=8
-24.30 ± 0.07 mUr (n=50)
FAO/IAEA
FEM
FSS
JSI
USGS

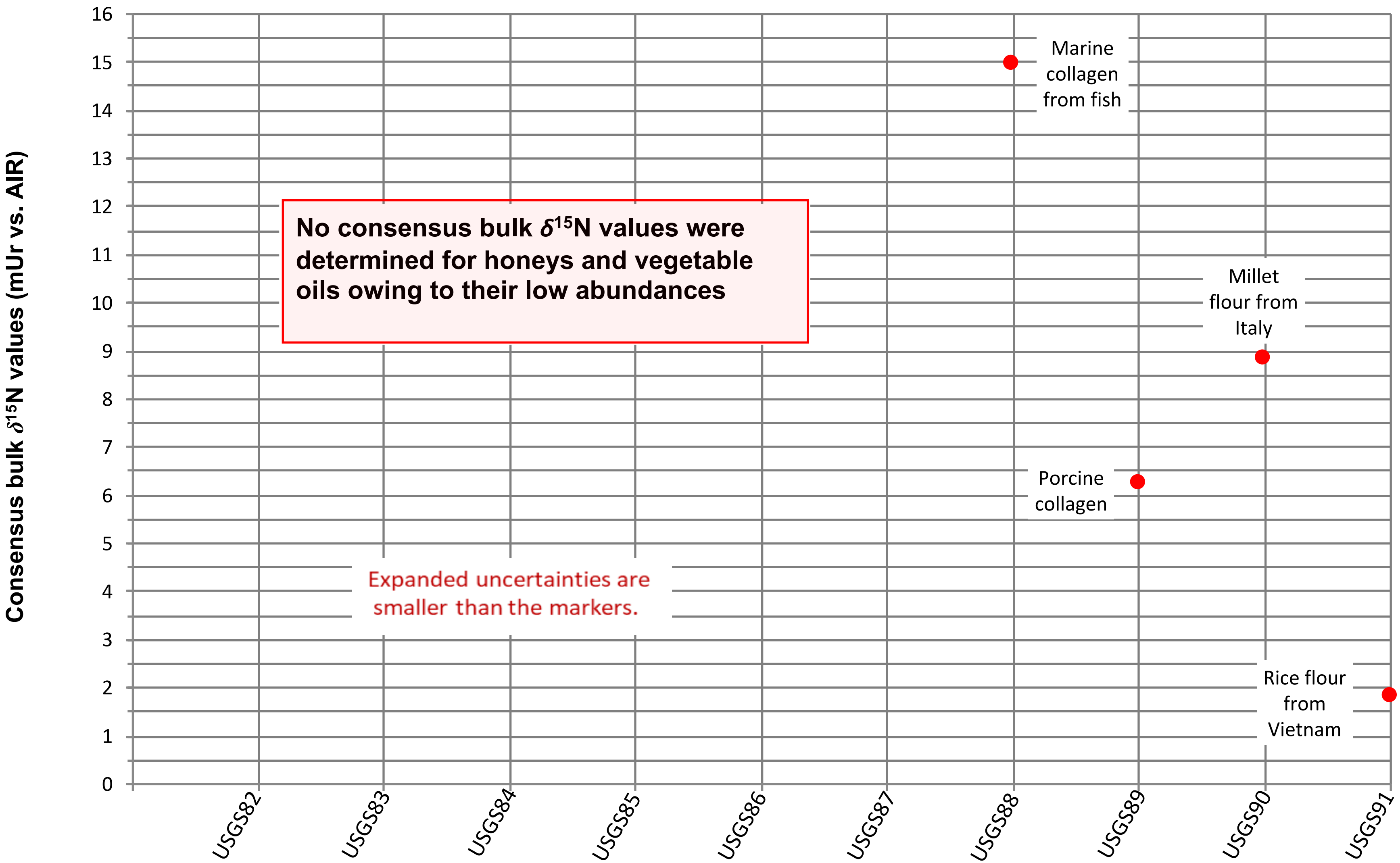
Results - δ^2H values USGS82 to USGS91



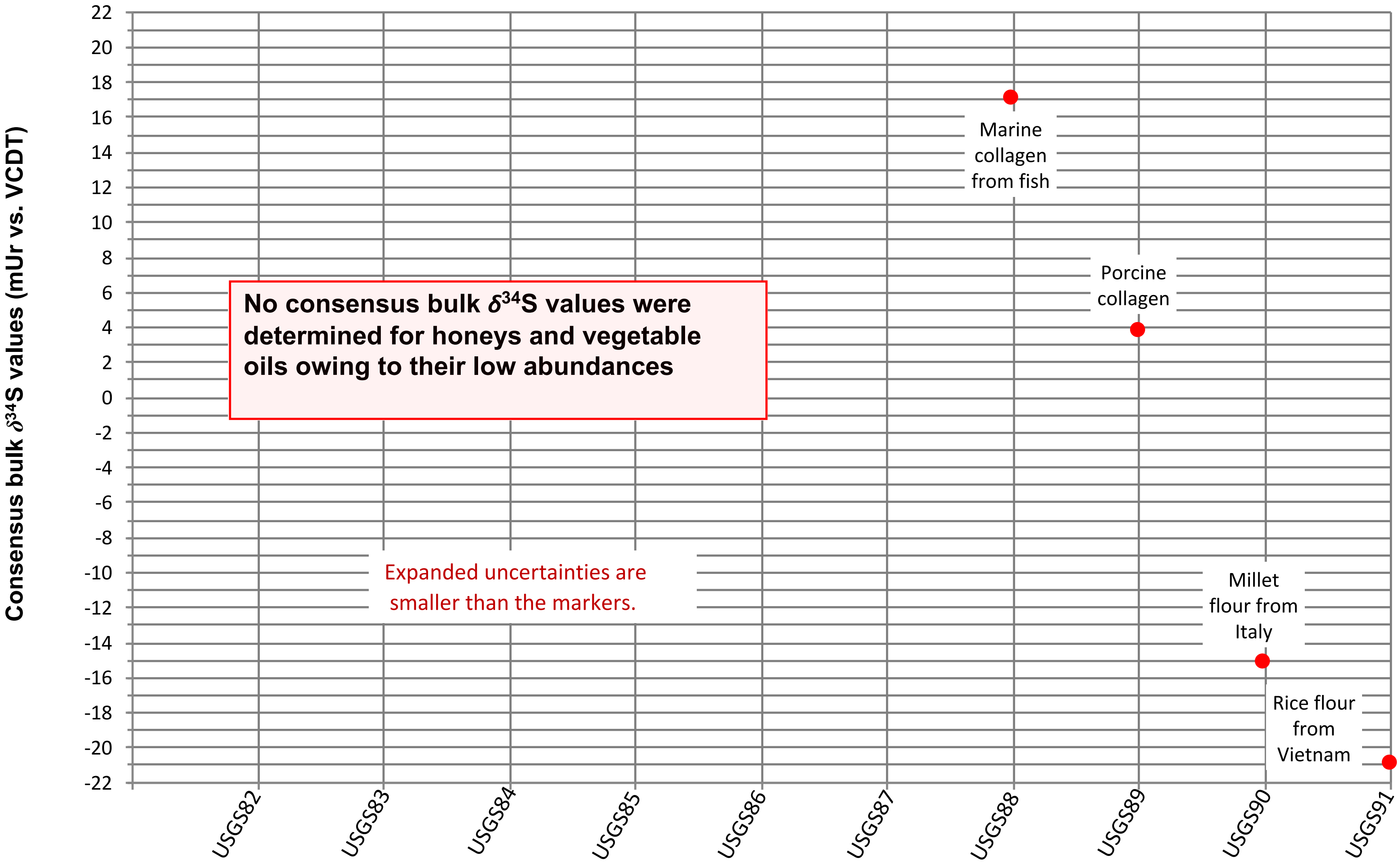
Results - $\delta^{18}O$ values USGS82 to USGS91



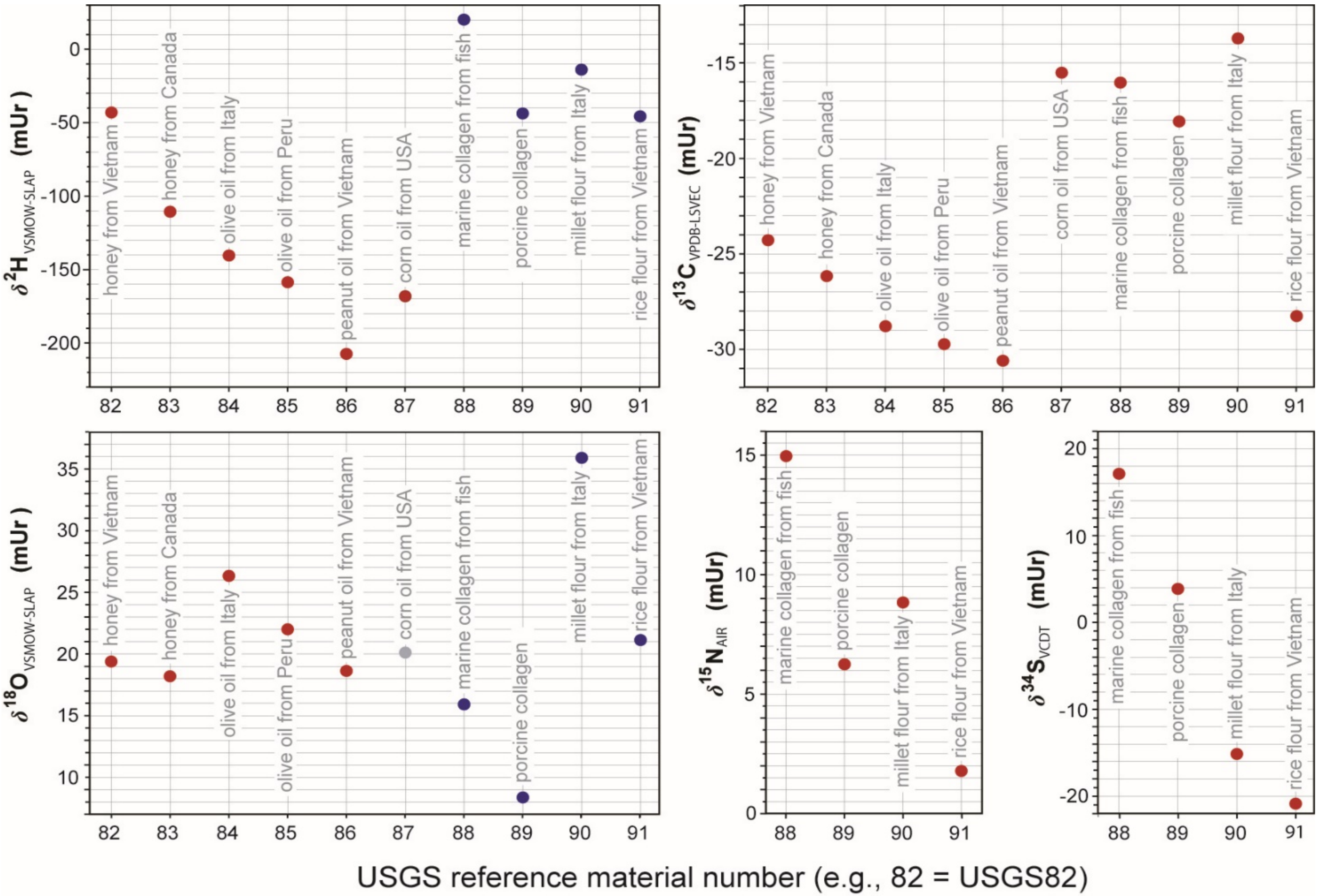
Results - δ^{15}N values USGS82 to USGS91



Results - δ^{34}S values USGS82 to USGS91



Results - isotopic values USGS82 to USGS91



Storage and future availability of RMs USGS82 through USGS91:

- All ″stock supplies″ of food matrix RMs are being stored under vacuum in hermetically sealed glass flasks in freezers at the USGS, Indiana University, and the Jožef Stefan Institute.
- End users will receive aliquots of RMs that are not sealed under vacuum.
- Aliquots of 1 mL of honeys and vegetable oils will be available in glass ampoules sealed under argon at Indiana University, and as 0.15 to 0.25-µL aliquots crimp-sealed without headspace in silver tubing by the USGS.
- Aliquots of 0.5 to 1 gram of dry, powdered food matrix RMs will be available to end users in screw-capped or crimp-sealed glass vials where the head- and pore space will be filled with air.
- Our manuscript is in review for the *Journal of Agricultural and Food Chemistry.*
- The new RMs USGS82 through USGS91 are anticipated to be released in 2020.

Adequate funding and patience are prerequisites for successful RM development

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THANK YOU FOR YOUR ATTENTION!