United States Geological Survey

Reston Stable Isotope Laboratory

Report of Stable Isotopic Composition

Reference Materials USGS82 and USGS83

(Hydrogen, Carbon, and Oxygen Isotopes in Honey)

These reference materials (RMs) are intended for normalization of stable hydrogen (δ^{2}H), carbon (δ^{13}C), and oxygen (δ^{18}O) isotope measurements of unknown honey and similarly-behaving hydrogen-, carbon-, and oxygen-bearing substances. A unit consists of 1 mL in a 2-mL glass ampule that is flame-sealed under argon. There is no limit on distribution. These RMs were prepared by A. Schimmelmann (Indiana University, Bloomington, Indiana). These RMs are not safe for human consumption and are strictly intended for laboratory use only.

Recommended Values: Stable hydrogen and oxygen isotopic compositions are expressed herein as delta values [1] relative to VSMOW (Vienna Standard Mean Ocean Water) on scales normalized such that the δ^{2}H and δ^{18}O values of SLAP (Standard Light Antarctic Precipitation) are –428 ‰ and –55.5 ‰, respectively [2,3,4]. Stable carbon isotopic compositions are expressed herein as delta values relative to VPDB (Vienna Peedee belemnite) on a scale normalized such that the δ^{13}C values of NBS 19 calcium carbonate and LSVEC lithium carbonate are +1.95 ‰ and –46.6 ‰, respectively [5]. Expanded measurement uncertainties at the 95 % confidence level are provided, and the coverage factors, *k*, used were (i) $k = 4$ for $\delta^{13}C_{VPDB\text{-}LSVEC}$ measurements, (ii) $k = 6$ for $\delta^{18}O_{VSMOW\text{-}SLAP}$ measurements, and (iii) $k = 9$ for $\delta^{2}H_{VSMOW\text{-}SLAP}$ measurements. Stable hydrogen-, carbon-, and oxygen-isotope delta values of USGS82 and USGS83 honeys with combined expanded uncertainties are given below. The hydrogen- and oxygen-isotope data were obtained by measurements of USGS82 and USGS83 sealed in silver tubes [6,7].

Reference	$\delta^{2}H_{VSMOW\text{-}SLAP}$	$\delta^{13}C_{VPDB\text{-}LSVEC}$	$\delta^{18}O_{VSMOW\text{-}SLAP}$	Data source
USGS82 Tropical Vietnamese honey	–43.1 ± 3.7 ‰	–24.31 ± 0.08 ‰	+19.44 ± 0.36 ‰	[7]
USGS83 Canadian Prairie honey	–110.5 ± 3.5 ‰	–26.20 ± 0.08 ‰	+18.20 ± 0.25 ‰	[7]

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Information Values: Hydrogen-, carbon-, and oxygen-mass fractions are provided as information values. Note that drying of honeys will decrease the moisture and affect the elemental mass fractions. These values were obtained by measurements of USGS82 and USGS83 sealed in silver tubes [6,7]. Uncertainties are standard deviations.

Reference	Element	Mass fraction	Data source
USGS82 Tropical Vietnamese honey	hydrogen	0.0717 ± 0.0006 (n = 6)	[7]
	carbon	0.3301 ± 0.0046 (n = 10)	[7]
	oxygen	0.5746 ± 0.0116 (n = 6)	[7]
USGS83 Canadian Prairie honey	hydrogen	0.0724 ± 0.0003 (n = 6)	[7]
	carbon	0.3412 ± 0.0047 (n = 10)	[7]
	oxygen	0.5714 ± 0.0068 (n = 6)	[7]

Technical coordination for these RMs was provided by Arndt Schimmelmann of Indiana University and Haiping Qi of the U.S. Geological Survey Reston Stable Isotope Laboratory (RSIL).

Expiration of Reference Values: The reference values for the isotopic compositions of USGS82 and USGS83 are valid until December 31, 2026, provided these RMs are stored in a freezer upon receipt and are handled in accordance with the instructions given in this Report of Stable Isotopic Composition (see "Instructions for Use"). A reference value is nullified if the RM is damaged, contaminated, or otherwise modified.

Sources of the RMs: This information is taken directly from Schimmelmann and others [7]. The Vietnamese honey (USGS82) was collected in late October 2018 directly from wild bee hives in the jungle 200 km west of Ho Chi Minh City, Kiên Giang Province (10°0′N 105°10′E). The 8 L of liquid honey were expedited to Indiana University where the honey was filtered twice through multiple layers of fine gauze (cheese cloth) at ambient temperature in November 2018. The original high moisture mass fraction of ~0.22 g g^{-1} (determined by weight loss after freeze-drying 4.8 g of honey) compromised the biochemical stability and would have increased the likelihood of spontaneous sugar fermentation. Honey should ideally contain a maximum of 0.20 g g^{-1} water [8]. Drying of Vietnamese liquid honey was accomplished by placing liter amounts of the filtered honey into pre-annealed Pyrex glass trays for three days each in December 2018 and January 2019 when the laboratory air's relative humidity was ~30 %. The Pyrex trays were shielded from dust by a cover made from cotton gauze. Re-weighing the trays with the partially dried honey enabled determination of the water loss. Subsequently, all Vietnamese honey was pooled into a single collapsible, airtight polyethylene container with a stopcock spout, where it was homogenized while being warmed gently to ~40 °C to reduce viscosity [9]. Two days of freeze-drying of an aliquot of the pooled liquid honey yielded a moisture loss of 0.14 g g^{-1}.

Honey from Saskatoon in Saskatchewan (USGS83), Canada, was purchased in December 2018 from *Three Foragers Bee Company* (https://www.threeforagers.ca/). The 6-kg supply of honey was produced exclusively from hives located in the prairie within a radius of 30 km of Saskatoon (52°08′N 106°41′W). The honey arrived at Indiana University as a solid. After liquefaction of the honey during gentle heating in a water bath at 45 °C, the honey was filtered through multiple layers of gauze (cheese cloth) at room temperature in December 2018. The liquid honey was pooled into a single collapsible, airtight polyethylene container with a stopcock spout where it was homogenized at ~40 °C. Two days of freeze-drying of an aliquot of the pooled liquid honey indicated a moisture loss of 0.094 g g^{-1}.

For long-term storage of more than 95 % of the supply, aliquots of both liquid honeys were transferred from their containers through spouts connected to Teflon tubing into pre-annealed 100-, 250-, 500-, and 1000-mL borosilicate glass flasks with pre-constricted necks while minimizing exposure to air. Filled glass flasks were individually attached to a vacuum line, each headspace was evacuated until incipient low-pressure boiling/outgassing at room temperature began, and each glass flask was sealed under vacuum with a glass-blowing torch. Both honeys solidified during cold storage. Honey supplies are being stored in freezers at Indiana University in Bloomington and the U.S. Geological Survey (USGS) in Reston, Virginia.

Maintenance of RM Report of Isotopic Composition: The U.S. Geological Survey RSIL will monitor these RMs and will notify the purchaser if substantive technical changes occur that affect their isotopic compositions.

Distribution and Stability: A distribution unit is available in amounts of 1 mL in a 2-mL glass ampule that has been flame-sealed under argon. The shelf life of unopened glass ampules of USGS82 and USGS83 stored in a freezer is five years. After opening of an ampule and exposing the honey to atmospheric oxygen and moisture, the remaining shelf life is two years as long as the honey is shielded from prolonged exchange with atmospheric moisture and kept in a freezer.

Instructions for Use: USGS82 and USGS83 can be interspersed among every 10–15 unknowns. Freezing will not eliminate slow oxidation, which will likely shorten the shelf life. After opening of any container of food matrix RMs, the remaining shelf life greatly depends on the handling and further storage of the material. For example, after opening a glass ampoule it is necessary to expeditiously transfer the liquid RM into another suitable container that can be closed tightly and stored at low temperature. In any case, exposure to atmospheric oxygen decreases the shelf life of RMs.

After opening a glass ampule with 1 mL of honey, the end user may find it difficult to efficiently transfer the viscous honey to another container while limiting the exposure of honey to room air. Instead, a compact, pea-sized ball of Parafilm M can be pressed onto the opening of the ampule for closure and can be temporarily removed for extracting aliquots of honey. Care must be exercised to avoid injury by the sharp glass rim. An additional layer of Parafilm M wrapped tightly around the ball and the upper part of the opened glass ampoule will further limit the exposure to room air during storage of honey. Parafilm M ball and wrapping do not need to be removed when warming honey in an opened ampoule to ~45 °C in air for re-homogenization.

Honeys sealed in silver tubing by the USGS offer several advantages because (i) silver metal has natural antimicrobial properties, (ii) samples are shielded from light and air, and (iii) uncertainties in weighing errors and potential contamination and fractionation by the end user are eliminated. Each crimp-sealed segment of silver tube contains honey with a bulk amount of hydrogen equivalent to the amount of hydrogen in 0.15 µL or 0.25 µL of reference water (available from the USGS). When using silver tube segments in an EA carousel, it can be advantageous to wrap the silver tube segment(s) in a tin or silver capsule to prevent jamming of the carousel and to ensure proper dropping of sample(s). The small amount of sample in silver tube segments precludes their use for $\delta^{34}S$ analysis.

Reporting of Stable-isotope-delta Values: The following recommendations are provided for reporting stable hydrogen-, carbon-, and oxygen-isotope data. It is recommended that:

- The δ^2H and $\delta^{18}O$ values of honey and similar hydrogen- and oxygen-bearing materials be expressed relative to VSMOW-SLAP on a scale where δ^2H_{SLAP} = –428 ‰ exactly or δ^2H_{SLAP2} = –427.5 ‰ [3,10].
- The $\delta^{13}C$ values of all carbon-bearing substances be expressed relative to VPDB-LSVEC on a scale such that the $\delta^{13}C$ values of NBS 19 calcium carbonate and LSVEC lithium carbonate are +1.95 ‰ and –46.6 ‰, respectively [3,5], even though LSVEC is no longer recommended as a RM for $\delta^{13}C$ measurement [11].
- Authors of new publications report delta values of international distributed (secondary) isotopic reference materials as though they had been interspersed among and used for normalization of unknowns, as appropriate for the measurement method. In this manner, measurement results can be adjusted in the future as analytical methods improve and consensus values of internationally distributed isotopic reference materials change.
- Reporting of delta values relative to PDB (Peedee belemnite) be discontinued [12].

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