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Sucrose certified reference material - UME CRM 1309 $\delta^{13}C_{VPDB}$ value assignment by EA-IRMS

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Abstract: A certified reference material (UME CRM 1309) of sucrose for carbon isotope ratio: $\delta^{13}C_{VPDB}$ (‰) value was produced according to the requirements of the ISO Guide 34:2009. The certified value with uncertainty was determined as -26.78 ± 0.81 ‰ (at k=2) by Elemental Analyzer Isotope Ratio Mass Spectrometry (EA-IRMS) method. Production and the certification processes include material processing, homogeneity study, stability studies (short-term and long-term), characterization study, statistical data evaluation and assignment of the property value and uncertainty. The $\delta^{13}C_{VPDB}$ certified value of sucrose was determined by an inter-laboratory comparison study among a network of seven competent laboratories.

Keywords*:* CRM, EA-IRMS; homogeneity; stability; characterization; assigned value; uncertainty; inter-laboratory comparison.

1. Introduction

Honey is a widely consumed food product having increasing commercial importance in recent years and it is sometimes adulterated with low-cost sugars. The quality parameters for honey was outlined in the Turkish Food Codex for honey published in the official newsletter by the Ministry of Food, Agriculture and Livestock [1], and one of them is concerning the adulteration of honey by sugar. Since, honey has a major place among the agricultural products consumed in Turkey, it is becoming more important to understand whether honey is adulterated or not.

The $\delta^{13}C_{VPDB}$ (‰) values vary from -22 to -33 in honey from C_3 plants and -10 to -20 in honey from C_4 plants [2]. Stable carbon isotope ratio (SCIRA) detects the natural abundance of the stable carbon isotopes and provides the isotope ratio between ^{12}C which is the most abundant in nature (99%) and its isotope ^{13}C, with low abundance 1%. This ratio reflects the photosynthetic cycle concerning the utilization and fixation of CO_2 by different plants [2]. The process of CO_2 fixation by plants according to one of the following three pathways described by Hatch, Slack & Johnson (1967, 1979): the Calvin and Benson cycle or C_3 cycle, occurred in most of the plants, that $\delta^{13}C_{VPDB}$ (‰) values range from -22 to -33, the Hatc Slack cycle or C_4 cycle occurring in a more limited number of plants that $\delta^{13}C_{VPDB}$ (‰) values range from -10 to -20, and the cycle of Crassulacean Acid Metabolism plants (CAM), such as cactus, pineapple etc. which are able to perform both cycle of CO_2 fixation with $\delta^{13}C_{VPDB}$ (‰) values ranging from -11 to -13.5, respectively [2,3,4]. Thus, carbon isotope ratios provide an important means of differentiating C3-derived sweeteners (beet) and C_4-derived sweeteners (cane, corn, HFCS) [5].

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Certified reference material (CRM) of sucrose is used as reference material for calibration/correction tool in $\delta^{13}C_{VPDB}$ (‰) measurements in honey. By determining $\delta^{13}C_{VPDB}$ (‰) values, honey samples can be identified as being adulterated with cheap sugar or not. It is not always possible to determine added sugars in honey by classical laboratory methods. Isotope ratio determination method is used to measure $\delta^{13}C_{VPDB}$ (‰) value and to identify the adulteration of honey. Both honey itself and its protein content (extracted from honey) are efficiently measured by IRMS for this purpose. $\delta^{13}C_{VPDB}$ values are expected to vary from -23‰ to -28 ‰ for unadulterated honey and -9 ‰ to -15 ‰ for adulterated honey [6, 7]. Therefore, in order to provide the proper CRM to field laboratories, a reference material such as sucrose with certified $\delta^{13}C_{VPDB}$ (‰) values within targeting the correct range is of extreme importance.

Certified reference materials (CRM) are used for validation purposes besides calibration, correction of raw measurement results and quality control. Use of CRMs is one of the most important tool for assuring measurement traceability and consequently the measurement quality. Use of CRM as it is outlined in ISO Guide 32 is also important as well as using matrix matching CRM. Use of matrix matching CRM in measurements serves several benefits and enhances measurement quality. Production and certification of these materials are planned according to the needs of field laboratories, and lack or cost of available CRMs.

Production and certification of sucrose CRM was carried out in accordance with the requirements of the ISO Guide 34 and 35 by using TUBITAK UME quality and technical infrastructure [8, 9]. The average value obtained from the accepted comparison study results has been used in the value assignment of the CRM. Homogeneity between units and stability tests were carried out according to ISO Guide 35:2006. Characterization strategy was planned to utilize several laboratories applying one or more independent methods as it is described in ISO Guide 34. Preliminary measurements, filling, labeling, homogeneity, short and long term stability tests were carried out at TUBITAK UME. Candidate reference material (sucrose) was sent to 6 laboratories for the measurement of the $\delta^{13}C_{VPDB}$ (‰) values.

2. Materials and Methods

2.1. Material

Sucrose, with purity of ≥ 99% was purchased from Sigma-Aldrich (CAS no: 57-50-1, cat. no: S7903).

2.2. Material Processing

Sucrose purchased from Sigma-Aldrich which was in powder form was homogenized with 3D mixer (Willy A. Bachofen AG Maschinenfabrik, TURBULA® T10-B, Switzerland) for 8 hours. Homogenized product was weighed and filled into the 5 ml amber glass bottles (total 200 bottles). Filling process was completed by filling CRM candidate as approximately 1 gram in each bottle, then capped bottles were stored at 18 ^{o}C in a temperature controlled cabinet until measurements for homogeneity, stability and characterization were performed

2.3. Homogeneity study

Homogeneity study between the units is performed to show that assigned value is valid for all produced units within the stated uncertainty. It is performed with the number of samples representing the whole batch. In this study, 20 units (10 spare) were selected by using random stratified sampling strategy and were reserved at 18 ^{o}C which was selected as a reference temperature for the study of homogeneity between units. Homogeneity tests were carried out by measuring 3 sub-samples under the repeatability conditions. The candidate CRM material was weighed about 200 µg in tin capsules for the determination of $\delta^{13}C_{VPDB}$ (‰) values.

The method used for these measurements was validated and the samples to be analyzed were introduced randomly to EA-IRMS instrument (Thermofinnigan MAT 253, Germany) in order to find out whether there was any trend arising from analytical and/or filling sequence or not.

Two reference materials, sucrose (NIST RM 8542) and L-glutamic acid (NIST RM 8573) were used for correction of $\delta^{13}C_{VPDB}$ values of raw data obtained from the candidate CRM measurements. Reference materials and samples were analyzed within the same run.

The data were evaluated statistically by regression analysis for the presence of any trend in analytical and/or filling sequence and no trend was found for candidate CRM at 95% and 99% confidence levels. Grubbs test was applied to all data for detecting outlier(s) at 95% and 99% confidence levels. No outlier was found.

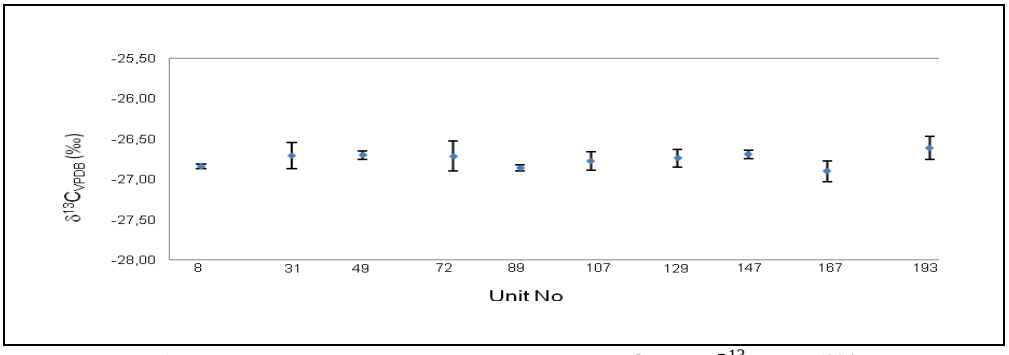

Figure 1. UME CRM 1309, sucrose unit number vs. $\delta^{13}C_{VPDB}$ (‰)

Table 1. Homogeneity data for UME CRM 1309, sucrose

Unit Number	Analytical Sequence			$\delta^{13}C_{VPDB}$ (‰)		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
8	11	6	16	-26.81	-26.86	-26.84
31	1	21	26	-26.74	-26.53	-26.86
49	17	27	22	-26.64	-26.75	-26.72
72	12	2	7	-26.79	-26.50	-26.85
89	8	18	28	-26.88	-26.81	-26.87
107	23	3	13	-26.91	-26.73	-26.69
129	29	9	4	-26.79	-26.81	-26.61
147	14	24	19	-26.65	-26.76	-26.67
167	10	5	25	-26.81	-26.84	-27.04
193	30	15	20	-26.75	-26.47	-26.62

Table 2. Uncertainty estimation of homogeneity study for UME CRM 1309

Reference Material	Average value $\delta^{13}C_{VPDB}$ (‰)	s_{wb} (‰)	s_{bb} (‰)
Sucrose (UME CRM 1309)	-26.75	0.11	0.06

Analysis of Variance (ANOVA) is a statistical tool used to estimate the uncertainty contribution from homogeneity of the material. All data were examined for normal distribution using Shapiro-Wilk test and visual histogram control before applying the one way ANOVA test. The candidate CRM showed normal distribution both on Shapiro-Wilk test and histogram control. The data for the homogeneity measurements are given in Table 1 and the homogeneity plots are given in Figure 1. Error bars in Figure 1 shows standard deviation of 3 replicate measurements for each tested unit. Uncertainty of homogeneity between units was calculated using one way ANOVA. Equation (1) is used for repeatability of method (s_{wb}) and equation (2) is used for the calculation of standard deviation between units (s_{bb}).

$$s_{wb} = \sqrt{MS_{within}} \quad (1)$$

Where,

MS_{within} : mean of square of variance within the unit

s_{wb} equals to "standard deviation" of the method as long as sub samples represent the whole unit.

$$s_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n}} \quad \text{where,} \quad (2)$$

$MS_{between}$: mean of square of variance between units
n : number of replicates per unit

The uncertainty value obtained from the homogeneity study for UME CRM 1309 is given as s_{bb} in Table 2.

2.4. Stability Study

Stability studies were carried out with the simulation of transfer conditions in the laboratory, considering environmental conditions that may occur during shipment to the user and storage conditions. One high and one low temperature points were selected.

2.4.1. Short Term Stability Study

Effect of shipment condition is tested on candidate CRM in short term stability study. Stability studies are performed with isochronous design which is cited in the ISO Guide 35 [9]. For the Short Term Stability (STS) test, two different temperatures (4˚C and 60 ˚C) and 4 different time points (1, 2, 3 and 4 weeks) were selected. 10 CRM samples were selected by randomly. 8 samples were subjected to the test temperatures for the specified time intervals and two are kept at reference temperature (+18˚C) and used as reference.

Binary samples were moved to reference temperature after completion of the test time. After completion of the test for all time points, all samples were analyzed at the same time. Three replicate measurements were performed from each unit by IRMS-EA under the repeatability conditions for $^{13}C/^{12}C$ isotope ratio delta values.

The data for each temperature point were first examined by single Grubbs test for both 95% and 99% confidence intervals to find out outliers. There was no outlier and trend after evaluation of results.

Values calculated for each time point were plotted against the time for the assessment of short term stability. The relationship between variables was analyzed in order to determine if any significant change exists in $\delta^{13}C_{VPDB}$ values with the testing time (regression analysis). All data for the short term stability study are given in Table 3 and plots are given in Figure 2. Uncertainty due to short term stability is calculated using equation (3). Maximum time for transfer is chosen as 4 weeks for the candidate CRM.

$$u_{lts} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \times t \quad (3)$$

where,

RSD : relative standard deviation
t_i : time point for each replicate
$\bar{t}$: mean of all time points
t : maximum time suggested for transfer (4 weeks)

Result of this study showed that the CRM can be transferred to end users without any cooling effort as soon as the ambient temperature does not exceed 60 ^{o}C and duration does not exceeding 4 weeks. u_{sts} at 60 °C calculated for 4 weeks is used as the contribution of short term stability to the CRM uncertainty.

Table 3. STS Data for UME CRM 1309, sucrose in 4 °C and 60 °C

Sequence order	Unit No	Time (week)	Temp. (°C)	$\delta^{13}C_{VPDB}$ (‰)	Sequence order	Unit No	Time (week)	Temp. (°C)	$\delta^{13}C_{VPDB}$ (‰)
52	34-1	0	18	-26.15	52	34-1	0	18	-26.15
54	34-2	0	18	-26.36	54	34-2	0	18	-26.36
51	34-3	0	18	-26.28	51	34-3	0	18	-26.28
49	109-1	0	18	-26.22	49	109-1	0	18	-26.22
50	109-2	0	18	-26.31	50	109-2	0	18	-26.31
53	109-3	0	18	-26.37	53	109-3	0	18	-26.37
4	1-1	1	4	-26.73	28	17-1	1	60	-26.56
11	1-2	1	4	-26.62	35	17-2	1	60	-26.37
17	1-3	1	4	-26.74	41	17-3	1	60	-26.43
9	188-1	1	4	-26.69	33	148-1	1	60	-26.41
16	188-2	1	4	-26.69	40	148-2	1	60	-26.44
23	188-3	1	4	-26.56	47	148-3	1	60	-26.53
12	97-1	2	4	-26.64	36	40-1	2	60	-26.34
13	97-2	2	4	-26.72	37	40-2	2	60	-25.78
20	97-3	2	4	-26.61	44	40-3	2	60	-26.54
3	151-1	2	4	-26.55	27	90-1	2	60	-26.60
7	151-2	2	4	-26.66	31	90-2	2	60	-26.60
14	151-3	2	4	-26.69	38	90-3	2	60	-26.47
8	113-1	3	4	-26.66	32	57-1	3	60	-26.66
1	113-2	3	4	-26.57	25	57-2	3	60	-26.56
10	113-3	3	4	-26.67	34	57-3	3	60	-26.42
18	127-1	3	4	-26.67	42	80-1	3	60	-26.64
22	127-2	3	4	-26.55	46	80-2	3	60	-26.48
19	127-3	3	4	-26.62	43	80-3	3	60	-26.51
21	46-1	4	4	-26.59	45	25-1	4	60	-26.71
15	46-2	4	4	-26.66	39	25-2	4	60	-26.48
2	46-3	4	4	-26.66	26	25-3	4	60	-26.37
24	173-1	4	4	-24.99	48	142-1	4	60	-26.29
6	173-2	4	4	-26.59	30	142-2	4	60	-26.90
5	173-3	4	4	-26.58	29	142-3	4	60	-26.49

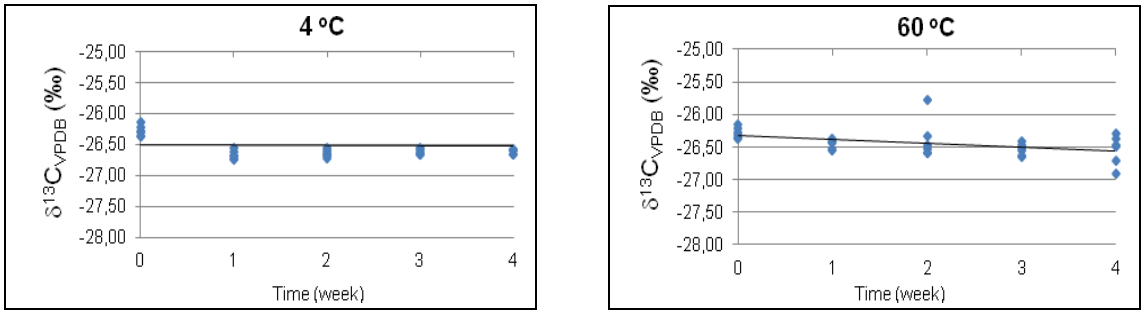

Figure 2. UME CRM 1309, short term stability plots: 4°C and 60 °C.

2.4.2. Long Term Stability Study

Shelf life of the produced CRM is determined by the long-term stability (LTS) studies. 25 ˚C was chosen as the test temperature for long term stability tests and totally 52 units (26 spare) of candidate CRM were reserved for this study. Samples were selected by randomly and stored at 25 ˚C for 12 months. Two units for each time point (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months) were

transferred from 25 ˚C to 18 ˚C (reference temperature) after completion of the test time. Three replicate measurements from each unit and were performed by IRMS-EA under the repeatability conditions for $^{13}C/^{12}C$ isotope ratio delta values.

The data was first examined by single Grubbs test for both 95% and 99% confidence intervals to find out outliers. One outlier for both 95% and 99% confidence intervals were found in this study. Since no technical reason can be found to reject this data, they were included in the LTS calculations. All data related with long term stability are given in Table 4 and plots are given in Figure 3.

Values calculated for each time point were plotted against the time for the assessment of LTS. The relationship between variables was analyzed in order to determine if there is any significant change exists or not in $\delta^{13}C_{VPDB}$ values with respect to the testing time (regression analysis). It was found that the slope was not significantly different from zero for CRM both in the 95% and 99% confidence intervals. Uncertainty contribution of long term stability, u_{lts}, was calculated using equation (5) for 5 years of shelf life at 25 °C.

$$u_{lts} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \times t \qquad (5)$$

where,

RSD : relative standard deviation obtained from all data in LTS

ti : time point for each replicate

$\bar{t}$: mean of all time points

t : shelf life suggested at 25 °C

Shelf life for CRM is set as 5 years after sales. Continuous post certification monitoring studies are going to be carried out in order to check the validity of the certified value over longer time.

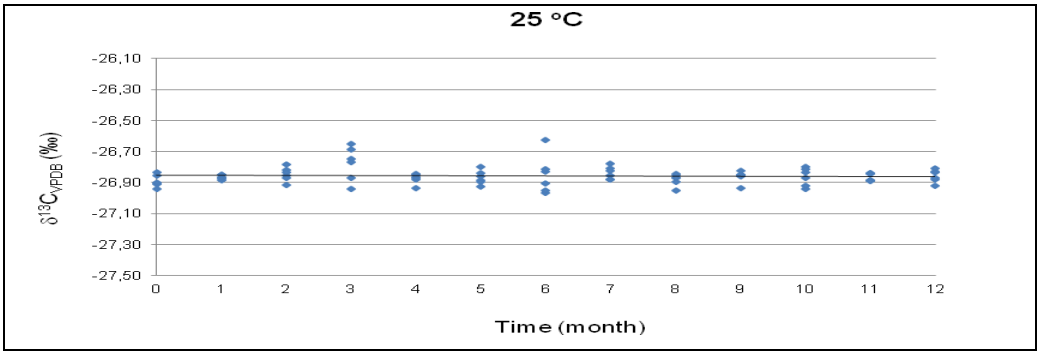

Figure 3. UME CRM 1309, sucrose long term stability plot

2.3. Characterization

Characterization and value assignment can be made in various ways in accordance with the ISO Guide 34 [8]. In this project, characterization was performed for a specifically defined measurand traceable to a specifically defined VPDB scale using a network of competent laboratories. Selection criteria for participating laboratories were:

- expertise in $\delta^{13}C_{VPDB}$ analysis
- having accreditation for the measurements of same or similar sample matrices
- Successful participation in proficiency testing schemes.

Candidate CRM (UME CRM 1309) was sent to 6 different national laboratories for the measurement of $\delta^{13}C_{VPDB}$ values. The $\delta^{13}C_{VPDB}$, ‰ assigned value (certificate value) and uncertainty value (k=2, 95 % confidence level) of CRM were found to be as -26.78 ‰ and 0.81 ‰, respectively.

Laboratories used validated methods in their measurements. 2 units were measured by each laboratory in two different days and the selection of these units was made randomly to represent all produced batch.

Table 4. LTS Data for UME CRM 1309UME CRM 1309 Sucrose

Sequence order	Unit No	Time (month)	Temperature (°C)	$\delta^{13}C_{VPDB}$ ‰
82	34-1	0	18	-26.84
84	34-2	0	18	-26.91
86	34-3	0	18	-26.86
88	109-1	0	18	-26.91
83	109-2	0	18	-26.94
85	109-3	0	18	-26.90
1	3-1	1	25	-26.85
25	3-2	1	25	-26.87
49	3-3	1	25	-26.88
37	195-1	1	25	-26.87
13	195-2	1	25	-26.86
61	195-3	1	25	-26.85
2	20-1	2	25	-26.79
26	20-2	2	25	-26.86
50	20-3	2	25	-26.87
38	178-1	2	25	-26.82
14	178-2	2	25	-26.92
62	178-3	2	25	-26.84
3	25-1	3	25	-26.69
27	35-2	3	25	-26.65
51	35-3	3	25	-26.94
39	158-1	3	25	-26.75
15	158-2	3	25	-26.87
63	158-3	3	25	-26.77
4	51-1	4	25	-26.86
28	51-2	4	25	-26.88
52	51-3	4	25	-26.94
40	145-1	4	25	-26.87
16	145-2	4	25	-26.85
64	145-3	4	25	-26.85
5	68-1	5	25	-26.80
29	68-2	5	25	-26.89
53	68-3	5	25	-26.90
41	125-1	5	25	-26.84
17	125-2	5	25	-26.92
65	125-3	5	25	-26.86
6	85-1	6	25	-26.83
30	85-2	6	25	-26.97
54	85-3	6	25	-26.81
42	108-1	6	25	-26.95
18	108-2	6	25	-26.91
66	108-3	6	25	-26.62
7	92-1	7	25	-26.78
31	92-2	7	25	-26.86
55	92-3	7	25	-26.88
43	102-1	7	25	-26.81
19	102-2	7	25	-26.88
67	102-3	7	25	-26.83
8	76-1	8	25	-26.87
32	76-2	8	25	-26.95
56	76-3	8	25	-26.85
44	120-1	8	25	-26.84
20	120-2	8	25	-26.90
68	120-3	8	25	-26.87
9	60-1	9	25	-26.94

Sequence order	Unit No	Time (month)	Temperature (°C)	$\delta^{13}C_{VPDB}$ ‰
33	60-2	9	25	-26.82
57	60-3	9	25	-26.85
UME CRM 1309 Sucrose				
Injection No	Unit No	Time (month)	Temperature (°C)	$\delta^{13}C_{VPDB}$ ‰
45	134-1	9	25	-26.86
21	134-2	9	25	-26.85
69	134-3	9	25	-26.86
10	43-1	10	25	-26.83
34	43-2	10	25	-26.87
58	43-3	10	25	-26.94
46	152-1	10	25	-26.92
22	152-2	10	25	-26.80
70	152-3	10	25	-26.82
11	26-1	11	25	-26.84
35	26-2	11	25	-26.88
59	26-3	11	25	-26.89
47	170-1	11	25	-26.89
23	170-2	11	25	-26.84
71	170-3	11	25	-26.85
12	11-1	12	25	-26.83
36	11-2	12	25	-26.83
60	11-3	12	25	-26.88
48	187-1	12	25	-26.92
24	187-2	12	25	-26.87
72	187-3	12	25	-26.81

From each unit of candidate reference material, 3 independent measurement results were reported by each laboratory. For candidate reference material, each laboratory reported 6 independent measurement results for two units on two different days with measurement uncertainties together with a summary of methods they used. Details of the reference materials used for calibration/correction were reported as the source of measurement traceability. All participating laboratories used EA-IRMS method in the characterization study. Details of reference materials traceable to VDPB scale used by participants in inter-laboratory comparison are given in Table 6. Results obtained from characterization study are given in Table 5 and characterization plots are given in Figure 4. Data obtained from characterization study revealed normal distribution and measurement uncertainties were calculated according to the "Guide to the Expression of Uncertainty in Measurements (GUM)" and "EURACHEM/CITAC Guide Quantifying Uncertainty in Analytical Measurement" documents [9]. The uncertainty of characterization study (u_{char}) was calculated by combining the results obtained by the participating laboratories and reported according to the ISO Guide 35 [10].

Characterization standard uncertainty (u_{char}) was calculated using equation (6) by taking into account the uncertainties and the standard deviation of the results reported by the participating laboratories.

$$u_{char} = \sqrt{\bar{u}_{labs}^2 + \left(\frac{SS}{\sqrt{n}}\right)^2} \quad (6)$$

where,

u_{char} : uncertainty arising from characterization study

u_{labs} : Mean of standard uncertainties reported by the participating laboratories

SS : Standard deviation of accepted means of participating laboratories
n : Number of laboratories with accepted results

Table 5. Characterization results for UME CRM 1309

		Mean Value, $\delta^{13}C_{VPDB}$ (‰)
Lab. No	**Technique**	**UME CRM 1309**
1	EA-IRMS	-26.70
2	EA-IRMS	-26.84
3	EA-IRMS	-26.90
4	EA-IRMS	-27.19
5	EA-IRMS	-26.64
6	EA-IRMS	-26.38
7	EA-IRMS	-26.79

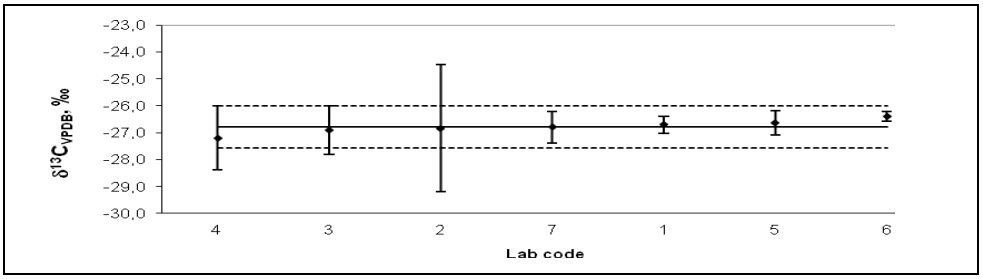

Figure 4. UME CRM 1309, sucrose characterization plot

Table 6. Reference Materials Used in Measurements for Traceability to VPDB scale

Lab No	Reference Material	Material Code	Traceable to reference material of the institute
1	Sucrose	IAEA-CH-6	IAEA
	L-glutamic acid	USGS 40	RSIL
2	Sucrose	IAEA-CH-6	IAEA
	Cellulose	IAEA-CH-3	IAEA
3	Caffeine	IAEA-600	IAEA
4	Cellulose	IAEA-CH-3	IAEA
5	Sucrose	IAEA-CH-6	IAEA
6	Cellulose	IAEA-CH-3	IAEA
7	Sucrose	IAEA-CH-6	IAEA
	Polyethylene	IAEA-CH-7	IAEA

2.4. Assigned Value and Uncertainty Determination

Data obtained in the characterization study were checked for normal distribution and outliers. Distributions were found to be normal and no outlier was detected. Mean value of all accepted characterization results is assigned as the property value of the reference material. Uncertainty of the CRM was calculated with combination approach of the uncertainty of characterization and uncertainty data that contribute to the homogeneity and stability assessments. The standard uncertainty of measurements were determined in accordance with Guide to the Expression of Uncertainty in Measurement (GUM) [11].

Formula (7) is used to calculate the combined expanded uncertainty of CRMs [11]:

$$U_{CRM} = k\sqrt{u_{char}^2 + u_{bb}^2 + u_{sts}^2 + u_{lts}^2} \quad (7)$$

Uncertainty value of CRM includes uncertainty contribution from characterization (u_{char}), homogeneity (u_{bb}), short term stability (u_{sts}) and long term stability (u_{lts}). Expansion of uncertainty value of CRM was done with a coverage factor (k=2) representing confidence level of approximately 95%. Certified value and uncertainty values are given in Table 7 and relative percent contribution of each component on uncertainty is given in Table 8.

Table 7. Certified value and uncertainity components of UME CRM 1309

CRM	Certificate value, $\delta^{13}C_{VPDB}$ (‰)	$U_{CRM,\ k=2}$ (‰)	u_{char} (‰)	u_{bb} (‰)	u_{sts} (‰)	u_{lts} (‰)
UME CRM 1309	-26.78	0.81	0.37	0.06	0.09	0.11

Table 8. Percent contribution of each parameter to u_{CRM}

CRM	$u_{char,\ rel}$ (%)	$u_{bb,\ rel}$ (%)	$u_{sts,\ rel}$ (%)	$U_{lts,\ rel}$ (%)
UME CRM 1309	85.0	2.0	5.5	7.5

3. Conclusion

Since honey is an expensive food, it can be easily adulterated by adding some cheap sugars. One of the best way to understand whether the honey is adulterated with cheap sugars or not is to determine the $\delta^{13}C_{VPDB}$ value of both honey and its protein. Laboratories working in this field need reference materials to calibrate/correct raw measurement results and validate their methods. Sucrose is one of the reference materials that is used for calibration/correction of raw data in $\delta^{13}C_{VPDB}$ (‰) values of honey measurements.

In this study, certified reference material of sucrose (UME CRM 1309) was produced and certified for its $\delta^{13}C_{VPDB}$ ‰ value and uncertainty by EA-IRMS method.

Assigned $\delta^{13}C_{VPDB}$ (‰) value of the material is -26.78 which is in the typical range for 95% of the plants living on earth. All the production and certification processes were carried out using TUBITAK UME infrastructure. Totally 7 competent/accredited Turkish laboratories including TUBITAK UME participated in inter-laboratory comparison study for characterization of the material. The certified value was determined as the mean of the accepted participant laboratory results.

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