Electronic Supplementary Information

Determination of sugar content in honey using LC-Raman and programmable pump-Raman methods

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Contents

1. Details of experimental apparatus

The diagram of the experimental apparatus is illustrated in Figure 1. Figure S1 shows supplementary information.

Fig. S1a displays the reservoir bottles of the running buffer and/or the sample solution(s). Only bottle A, containing H_2O, was used in the LC-Raman measurement, whereas both bottles A (containing H_2O) and B (containing 1000 mM aqueous solution of sugar) were used in the PP-Raman measurement.

Figs. S1b and c show the components of the HPLC setup. Fig. S1b depicts the pump unit (PU-4180-LPG, JASCO), which includes a degasser and mixing unit. The mixing unit was used in the PP-Raman measurement, but not in the LC-Raman measurement, as only one solvent was used. Fig. S1c is the UV-Vis colorimeter (UV-4075, JASCO) used to record the chromatogram.

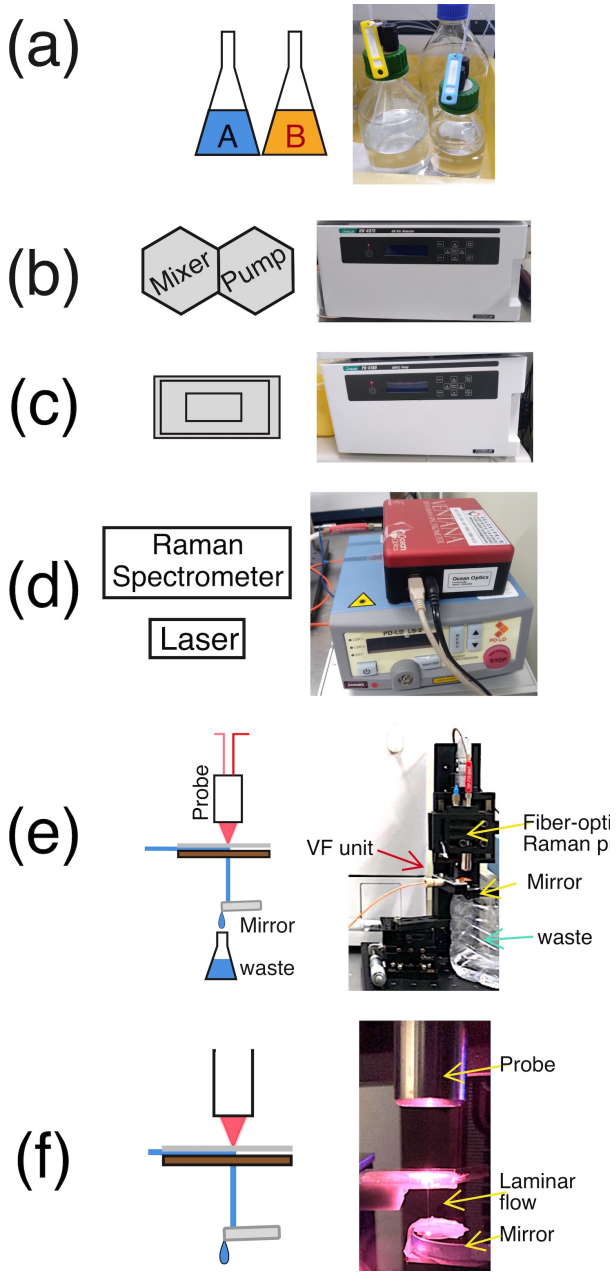

Figure S1 Results of concentration dependence of Raman spectrum of glucose (A), maltose (B), sucrose (C), and trehalose (D). Left panel shows a singular value plot (inset: IND factor up to 10 components), and reconstructed vectors of temporal (c) and spectral (d) components.

Fig. S1d displays the 785 nm cw laser (LS-2-785, PD-LD) and monochromator (Ventana, Ocean Optics). The monochromator is a package that comprises a 1024 × 32 pixel CCD detector (Hamamatsu S11510-1006 back-thinned NIR-enhanced CCD), a transmission grating (1600 gr/mm), and optimized 600 μm-fiber input (NA 0.39) with high throughput (F/1.3). The spectral resolution is 10 cm^{-1} at 810 nm.

This monochromator covers the Raman signals at 800 – 940 nm and is optimized for the 785 nm-excited Raman measurement.

Fig. S1e illustrates the vertical flow (VF) unit, and Fig. S1f provides the closer view. The HPLC outlet is connected to the VF unit via a standard HPLC fitting (UNF10-32). The sample liquid is spout from a pinhole of the VF unit and forms a vertical laminar flow. The Raman excitation beam is introduced into the laminar flow from the pinhole. The excitation beam and Raman signals are confined to the liquid column when the total reflection condition is satisfied. While the backward scattering Raman signal return to the pinhole, the forward scattering component is reflected by a mirror at the end of the laminar flow and returns to the pinhole. The Raman signals exit from the pinhole of the VF unit, collected by the Raman probe, delivered to the monochromator, and then detected. Thus, the VF method prevents the Raman signal from dissipating, improving the efficiency of the Raman signal generation and collection, and enhancing sensitivity in Raman spectroscopy.

2. Concentration dependence of Raman spectra of glucose, maltose, sucrose, and trehalose

We performed a programmable pump-Raman experiment to investigate the effect of sugar concentration on the intensity pattern of Raman spectrum. The sugar concentration was changed from 0 to 1000 mM and the Raman spectrum was continuously recorded. Singular value decomposition (SVD) analysis was performed on the 2D Raman data, and the SV plot (Figs. S2(a1-d1)) showed that the data involve more than four significant components in each case.

Two components were found to include the linear concentration dependence of the Raman spectra of the solvent and solute. The third component was investigated to identify any nonlinear concentration dependence, which would appear in the third component if present. The third component showed no remarkable concentration-dependent change in the Raman spectrum. This indicated that the intensity pattern of the Raman spectrum of each sugar changed in a concentration-dependent manner below 1000 mM, and other significant nonlinear dependence was absent.

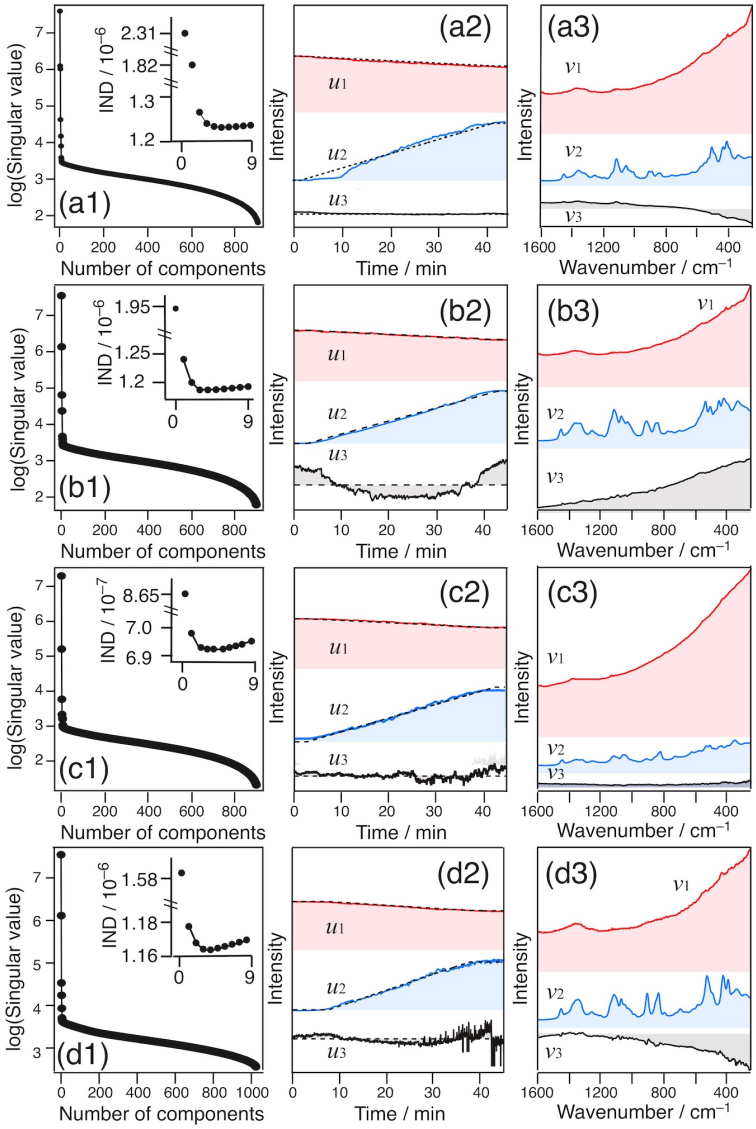

Figure S2 Results of concentration dependence of Raman spectrum of glucose (A), maltose (B), sucrose (C), and trehalose (D). Left panel shows a singular value plot (inset: IND factor up to 10 components), and reconstructed vectors of temporal (c) and spectral (d) components.

Three kinds of temporal (a2–d2) and spectral components (a3–d3) were reconstructed (Fig. S2). The reconstruction was conducted, in which the first and second vectors in the temporal matrix reproduced the expected linear behavior based on the programmed functions (see 2. Experimental for details). *u*1 (Fig. S2[a2–d2]; H_2O is 100% at 0–2 min, decreases down to 83% in 40 min, and keeps this value at 40–45 min) indicates the temporal behavior of the solvent. Accordingly, *v*1 (Fig. S2[a3–d3]) is consistent with the spectral pattern of the aqueous solvent (Carey & Korenowski, 1998). *u*2 represents the time-dependent linear increase in sugar concentration (Fig. S2[a2–d2]). Accordingly, *v*2 exhibits the Raman bands of each sugar (Fig. S2[a3–d3]). The spectral pattern of each sugar is consistent with that of the model compound in Fig. 2d. *u*3 and *v*3 show no concentration-dependent change in the Raman spectrum, indicating that the intensity pattern of the Raman spectrum of each sugar changes in a concentration-dependent manner in the range of 0–1000 mM, and other nonlinear dependence is not remarkable.

In this analysis, we disregarded the fourth and later components in the SV plot. Inset of Fig. S2 [a1–d1] shows the IND parameter (Mallnowski, 1977). The number of significant components and the fraction of the singular value of each component in the total sum of SV (SV%) are as follows:

six in glucose (SV% = 92.4, 2.9, 2.4, 0.10, 0.035, 0.019)

five in maltose (93.9, 3.7, 0.17, 0.063, 0.012)

five in sucrose (97.8, 0.79, 0.029, 0.011, 0.0083)

four in trehalose (92.2, 3.4, 0.089, 0.046)

Accordingly, the fraction of the lost information by omitting the fourth and later components is 0.2% in glucose, 0.1% in maltose, 0.1% in sucrose, and 0.1% in trehalose. Hence, the rejection of the fourth and later components leads to the loss of information and derives an error as small as 0.2% or less to the following discussion.

3. Result of regression analysis

Table S1 Parameters obtained from regression analysis

Analyte	Retention time	Coefficient of Equation† upper: a ± SD_a lower: b ± SD_b	R^2	LOQ	LOD
Fructose		9649.5 ± 20161.5 1894.9 ± 50.0	0.996	106.4 ± 2.5 mM (19.2 ± 0.5 mg mL^{-1})	35.1 ± 0.8 mM (6.3 ± 0.2 mg mL^{-1})
Glucose		17231.1 ± 8132.4 1749.5 ± 20.2	0.999	46.5 ± 0.5 mM (8.4 ± 0.1 mg mL^{-1})	15.3 ± 0.2 mM (2.8 ± 0.1 mg mL^{-1})
Sucrose		9137.2 ± 28605.1 3596.4 ± 70.9	0.998	79.5 ± 1.4 mM (27.1 ± 0.5 mg mL^{-1})	26.2 ± 0.5 mM (9.0 ± 0.2 mg mL^{-1})
Maltose		-20645.1 ± 14875.2 2686.4 ± 36.9	0.999	55.4 ± 0.7 mM (18.9 ± 0.3 mg mL^{-1})	18.3 ± 0.2 mM (6.3 ± 0.1 mg mL^{-1})
Trehalose		-26196.2 ± 11629.4 4540.5 ± 28.8	1.000	25.6 ± 0.2 mM (8.8 ± 0.1 mg mL^{-1})	8.5 ± 0.1 mM (2.9 ± 0.1 mg mL^{-1})

† $y = (a \pm \mathrm{SD_a}) + (b \pm \mathrm{SD_b})x$.

4. Contracted sugar analysis of honey: Mass spectrometry

Mass spectrometric analyses were performed by the Center for Advanced Instrumentation and Department of Applied Chemistry at National Yang Ming Chiao Tung University, Hsinchu, Taiwan, R.O.C. Mass spectra of honey solutions at pH 7.5 were acquired by direct infusion (2 μL). ESI(−) MS experiments were conducted using a Micromass Quattro triple quadrupole mass spectrometer (Waters, USA) equipped with an electrospray ionization (ESI) source operating in negative ion mode. The parameters of ESI(−) were set as follows: ion spray voltage of 4.0 kV, capillary temperature of 200°C, and sheath gas flow rate of 6 L/min. The mass spectra were collected over the mass range of m/z 50–1500 at a resolving power of 40,000. The collected data were analyzed using Compass DataAnalysis 4.1 (Bruker, Germany).

As shown in Fig. S4, the peak appears at 179.0572 and 341.1105. These peaks are considered as the peaks of anionic species $[M\text{-}H]^-$ of compounds with MW (M) of 180.0650 and 342.1183, respectively. Given that the monoisotopic mass of C, H, and O was 12.0000, 1.0078, and 15.9949, respectively, the calculated values of M of the monosaccharide ($C_6H_{12}O_6$) and disaccharide ($C_{12}H_{22}O_{11}$) are 180.0630 and 342.1155, respectively. M of the anionic form of each species match the observed peaks. That is, the result of MS analysis supports our conclusion that honey involves monosaccharide(s) and disaccharide(s).

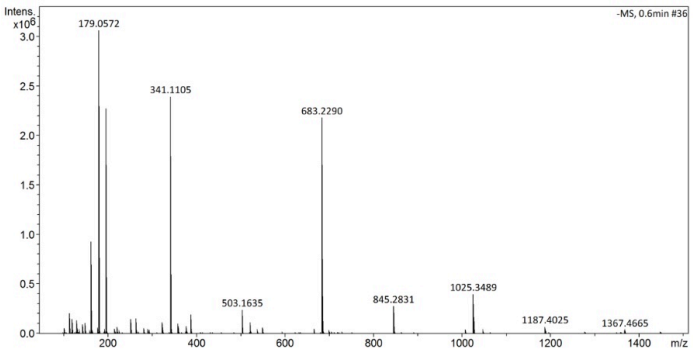

Figure S3 Result of mass spectrometry

5. Estimation of volume change upon mixing of two solutions

In the PP-Raman experiment, two liquids, namely, pure water (A) and sugar solution (B), are mixed at different fractions, and the concentration of the sugar is changed continuously. The volume of the mixed solution (V_{mix}) is not equal to the sum of the volume of the two solutions (V_A and V_B, respectively). The difference of the volume (Δvolume) between the real value (V_{mix}) and that calculated from the sum of the volumes of A and B ($V_A + V_B$) [Δvolume = $V_{mix} - (V_A + V_B)$] is evaluated by using the following procedure.

We consider the density to calculate the weight of two solutions S_A (0.997 [g cm^{-3}] for H_2O (Dean, 1999)) and S_B ($d_{1000\ mM}$ [g cm^{-3}] for 1000 mM sugar aqueous solution). 1 cm^3 of S_A contains 0.997 g of H_2O; S_B contains $10^{-3}\,m$ [g] of sugar [m is the molecular weight] and ($d_{1000mM} - 10^{-3}m$) [g] of H_2O.

When A and B are mixed at the volume fraction of (1–b) and b ($0 \le b \le 1$), respectively, the weight [g] of H_2O (w_{H_2O}) and sugar (w_{sugar}) in the mixture are given as follows:

$$w_{H_2O} = 0.997(1-b) + b(d_{1000\ mM} - 10^{-3}m)$$

$$w_{sugar} = 10^{-3}bm$$

The weight percent concentration of the sugar (c_w) is presented as follows:

$$c_w = \frac{w_{sugar}}{w_{sugar}+w_{H2O}} \times 100.$$

The c_w dependence data of the density of the sugar solution ($d_{solution}$) is given for maltose and trehalose (Gharsallaoui, Rogé, Génotelle, & Mathlouthi, 2008). The literature data are fitted with the third-order polynomial, and the fitted curve is used to obtain the density of the solution ($d_{solution}$) at the weight concentration of c_w. In addition, $d_{solution}$ is given for

the w/v% concentration (c_v) of glucose, fructose, and sucrose (Darros-Barbosa, Balaban, & Teixeira, 2003). c_v is converted to c_w by considering $d_{solution}$, and $d_{solution}$ at c_w was obtained.

The volume of the mixture ($V_{solution}$) is given as follows:

$$V_{solution} = (w_{H2O} + w_{sugar}) / d_{solution}.$$

Fig. S3 shows Δvolume upon mixing S_A and S_B at the fraction of B (or b). The volume change is up to −0.15% upon the mixing of the equivolume of S_A and S_B, and this value derives the maximum error of the molar concentration of sugar (c_M).

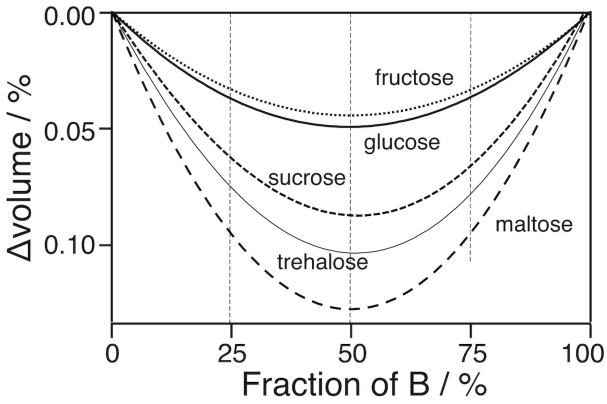

Figure S4 Calculated plot of volume change upon mixing of H_2O and aqueous solution of sugars.

References

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