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Method of Test for $\delta^{13}C$ of Saccharides in Honey

1. Scope

This method is applicable to the determination of stable carbon isotope ratio ($\delta^{13}C$) of saccharides in honey.

2. Method

Saccharides are separated by liquid chromatograph (LC) and oxidized under high temperature by sodium persulfate and phosphoric acid solution. The $\delta^{13}C$ of carbon dioxide are determined by stable isotope ratio mass spectrometer (IRMS).

2.1. Equipment

2.1.1. Liquid chromatograph/isotope ratio mass spectrometer (LC/IRMS)

2.1.1.1. Liquid chromatograph: Ultimate 3000, or an equivalent product.

2.1.1.2. Continuous flow interface: Conflo IV, or an equivalent product.

2.1.1.3. Continuous flow interface for liquid chromatography: LC IsoLink, containing flow injection analysis (FIA) mode and on-column mode, or an equivalent product.

2.1.1.4. Stable isotope ratio mass spectrometer (IRMS): Delta V Advantage, or an equivalent product.

2.2. Chemicals and gas

Trehalose (Note), turanose (Note), raffinose pentahydrate (Note), sodium persulfate, and phosphoric acid (85%), GR grade;

Deionized water, resistivity ≥ 18 MΩ·cm (at 25℃);

Helium and carbon dioxide, purity > 99.9995%;

IRMM-BCR-657 ($\delta^{13}C_{VPDB}$: -10.76‰) and IAEA-601 ($\delta^{13}C_{VPDB}$: -28.81‰), reference material.

Note: Trehalose, turanose and raffinose pentahydrate are regarded as reference material after being calibrated by elemental analyzer-isotope ratio mass spectrometer (EA-IRMS). Other reference materials are allowed to use depending on the range of $\delta^{13}C$ of samples.

2.3. Apparatus

2.3.1. Centrifuge tube: 50 mL, PP.

2.3.2. Sample vial: 2 mL.

2.4. Reagents

2.4.1. 20% sodium persulfate

Dissolve 100 g of sodium persulfate with deionized water to 500 mL.

2.4.2. 1.5 M phosphoric acid

Transfer 46 mL of phosphoric acid into a 500 mL volumetric flask and dilute with deionized water to volume.

2.5. Standard solution preparation

Transfer about 2.4 mg of each calibrated trehalose, turanose, raffinose pentahydrate, IRMM-BCR-657, and IAEA-601 accurately weighed into a 20-mL volumetric flask, dissolve and dilute with deionized water to volume. When to use, transfer 1 mL of solution into a sample vial and screw the cap after infusing with helium for 30 seconds as the standard solution.

2.6. Sample preparation

Transfer about 7 mg of the sample accurately weighed into each 20-mL volumetric flask, dissolve and dilute with deionized water to volume. When to use, transfer 1 mL of solution into a sample vial and screw the cap after infusing with helium for 30 seconds as the sample solution. Each sample should replicate 3 times.

2.7. Standard curve

Inject standard solutions into the LC-IRMS under FIA mode separately and operate according to the following conditions. Establish the standard curve of saccharides by the practiced values of $\delta^{13}C$ vs. the certified values (calibrated values for trehalose, turanose and raffinose pentahydrate) of $\delta^{13}C$.

LC-IRMS operating conditions (Note 1):

Column: Rezex™ RCM-Monosaccharide Ca^{2+}, 7.8 mm i.d. × 30 cm.

Column temperature: 55℃.

Mobile phase: deionized water.

Flow rate: 0.3 mL/min.

Injection volume: (1) FIA mode: 15 μL.

(2) on-column mode: 50 μL.

Pressure of reference gas (CO_2): 0.8 bar.

Pressure of carrier gas (He): 1.5 bar.

Pressure of purge gas (He)：1.3 bar.

Reactor temperature: 99.9℃.

oxidation reagent: 20% sodium persulfate and 1.5 M phosphoric acid.

Flow rate of oxidation reagent: 0.05 mL/min.

Background signal of oxygen (*m/z* 32) (with the signal amplification ratio 3×10^8): 10～15 V(Note2).

Detection ions: *m/z* 44, 45, 46.

Note: 1. All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2. The background signal of oxygen represents the oxidation potential, which depends on the flow rate of the 20% sodium persulfate. The flow rate of the reagent can be adjusted to the optimum value according to the actual situation.

2.8. $\delta^{13}C$ of saccharides

The sample solution was analyzed by LC-IRMS under on-column mode according to the conditions of Section 2.7. Calculate the calibrated $\delta^{13}C$ of monosaccharides (fructose and glucose), disaccharides and tri-saccharides in samples by the standard curve.

2.9. $\delta^{13}C$ of protein

Analyze the $\delta^{13}C$ of protein in samples according to the “Method of Test for C4 plant sugars in Honey (TFDAF0030.00)”.

2.10. Reference benchmark for adulteration

Calculate the $\delta^{13}C$ differences ($\Delta\delta^{13}C$) between each compound. Determine whether the sample may be adulterated according to the reference benchmark of the Coordinated Control Plan of honey in EU (listed in attached table).

Remark

Though this method can be used as a test of honey adulteration, the result should be determined comprehensively with the findings of investigations. Since the value of $\delta^{13}C$ may be differ from geographical origins, nectar sources, climates and environments.

References

1. Elflein, L. and Raezke, K. P. 2008. Improved detection of honey adulteration by measuring differences between $^{13}C/^{12}C$ stable carbon isotope ratios of protein and sugar compounds with a combination of elemental analyzer – isotope ratio mass spectrometry and liquid chromatography – isotope ratio mass spectrometry ($\delta^{13}C$-EA/LC-IRMS). Apidologie 39: 574-587.
2. Aries, E., Burton, J., Carrasco, L., De Rudder, O. and Maquet, A. 2016. Scientific support to the implementation of a Coordinated Control Plan with a view to establishing the prevalence of fraudulent practices in the marketing of

honey. Results of honey authenticity testing by liquid chromatography-isotope ratio mass spectrometry. JRC technical reports: JRC104749.

3. Taiwan Food and Drug Administration. 2017. Method of Test for C4 Plant Sugars in Honey (TFDAF0030.00). Published, Nov 14, 2017.

Reference chromatogram

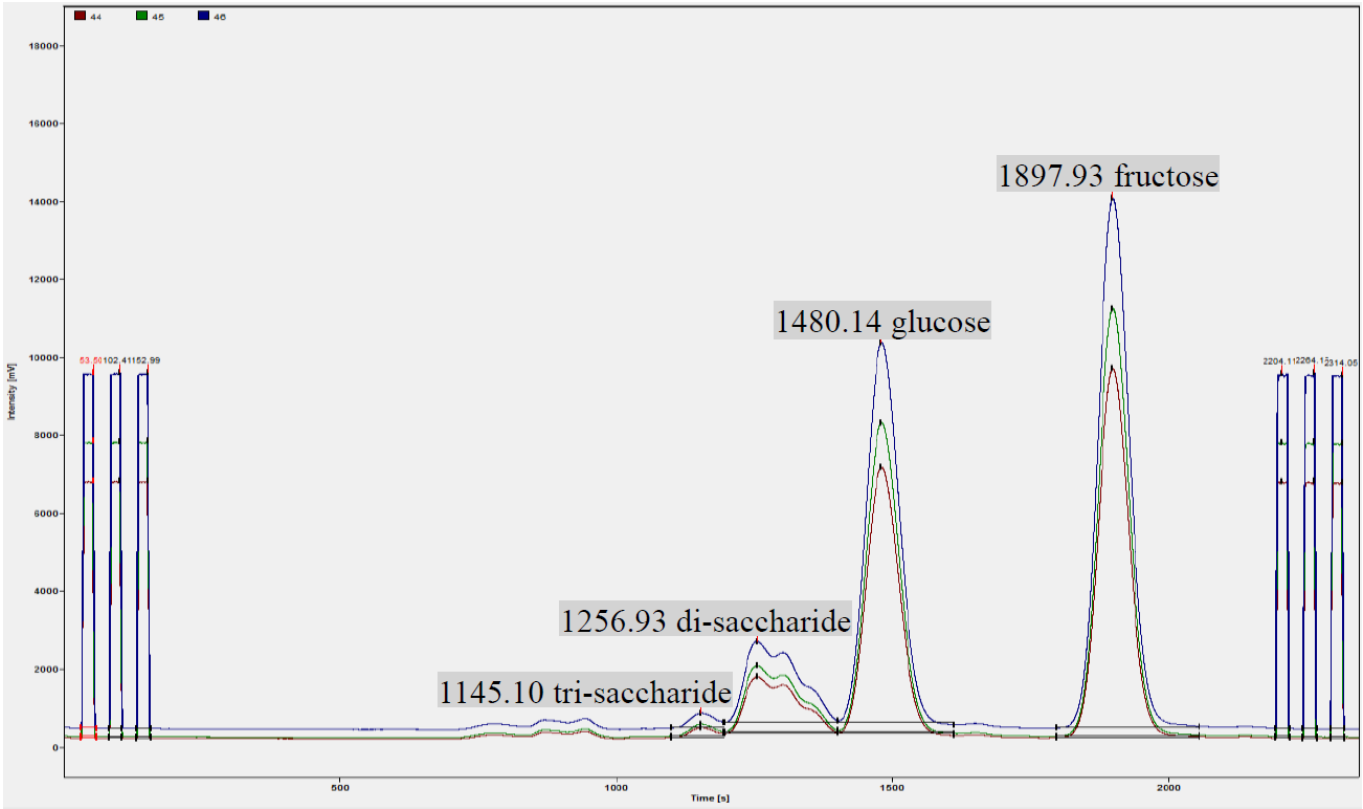

Figure. LC-IRMS chromatogram of saccharides in honey.

Table. Reference benchmark of the Coordinated Control Plan of honey in EU

Purity parameter of honey*	Proposed limit
$\triangle\delta^{13}C_{fru\text{-}glu}$	± 1‰
$\triangle\delta^{13}C_{max}$	
$\triangle\delta^{13}C_{fru\text{-}ds}$	
$\triangle\delta^{13}C_{fru\text{-}ts}$	
$\triangle\delta^{13}C_{fru\text{-}p}$	
$\triangle\delta^{13}C_{glu\text{-}ds}$	± 2.1‰
$\triangle\delta^{13}C_{glu\text{-}ts}$	
$\triangle\delta^{13}C_{glu\text{-}p}$	
$\triangle\delta^{13}C_{ds\text{-}ts}$	
$\triangle\delta^{13}C_{ds\text{-}p}$	
$\triangle\delta^{13}C_{ts\text{-}p}$	
Percent peak area of oligosaccharides	< 0.7%

*fru as fructose; glu as glucose; ds as disaccharide; ts as tri-saccharide; p as protein. (Aries et al., 2016)