

6/25/51

991.41

## C-4 Plant Sugars in Honey

### Internal Standard

### Stable Carbon Isotope Ratio Method

#### Action

Method can be used to resolve uncertainty in interpreting  $\delta^{13}\text{C}$  values between  $-23.4$  and  $-21.5$  ‰ (for citrus honey,  $-21.9$  and  $-20.0$ ‰). However, method is applicable to honey with any  $\delta^{13}\text{C}$  value. (See ref. 1 for supporting data.)

#### Method Performance:

Range 2.1 - 13.6%

$s_r = 1.25 - 2.69$ ;  $s_R = 1.97 - 2.69$ ;  $RSD_r = 9.22 - 90.9$ ;  $RSD_R = 14.5 - 92.6$

#### A. Principle

Stable carbon isotope ratio value for protein isolated from a honey provides standard to which stable carbon isotope ratio value of whole honey is compared.

#### B. Apparatus

(a) *Centrifuge*: With horizontal 4-head rotor for 50 ml tubes, to provide 1500 x g.

(b) *Isotope ratio mass spectrometer*- VG 602E, or equivalent.

#### C. Reagents

(a) *Tungstic acid, sodium salt*- 10% aqueous solution.

(b) *Sulfuric acid*- 0.67 (N) dilute 1.88 mL sulfuric acid to 100 mL.

#### D. Determination

(a) *Honey*- Determine  $\delta^{13}\text{C}$  of honey test portion as in 978.17.

(b) *Protein*- If appreciable amounts of solid matter are present in the

✓ honey, it must be strained through 100 - 150 mesh (nylon stocking material is excellent); ~~since~~ any insoluble material heavier than water will contaminate the protein precipitate.

Add 4 mL H<sub>2</sub>O to 10 - 12 g honey in clear 50 mL centrifuge tube; mix well. Add 2.0 ml 10% sodium tungstate solution and 2.0 ml 0.67N H<sub>2</sub>SO<sub>4</sub> to a small test tube, mix, and immediately add to honey solution; mix well. Swirl tube in *ca* 80° water bath until visible floc forms, with clear supernate. If no visible floc forms, or the supernate remains turbid, add acid in 2 ml increments, repeating heating between additions.

Fill tube with water, mix contents and centrifuge 5 min at 1500 x *g*, and decant supernate. Repeat washing, mixing, ~~and~~ centrifuging steps 5 times with *ca* 50 ml portions of water, thoroughly dispersing pellet each time.

Place an appropriate amount of the protein in a ceramic combustion boat similar to that used for honey samples. Combust protein samples by same method used for honey. If necessary to hold for later isotope ratio analysis, either transfer (Pasteur pipet) washed pellet with minimum of water to small vial, cap and place in boiling water for 2 min, or dry protein for at least 3 h in *ca* 75° oven. °°

✓ Calculate ~~the~~ apparent C-4 sugar content as follows:

$$\% \text{ C-4 sugars} = \{[\delta^{13}\text{C}_P - \delta^{13}\text{C}_H] / [\delta^{13}\text{C}_P - (-9.7)]\} \times 100$$

where  $\delta^{13}\text{C}_P$  and  $\delta^{13}\text{C}_H$  are  $\delta^{13}\text{C}$  values,  $\bar{E}$ , for protein and honey respectively; and -9.7 is the average  $\delta^{13}\text{C}$  value for corn syrup, ‰.

Report negative values from this calculation as 0%.

Sample is considered to contain significant C-4 sugars only at or above a value of 7%.

Ref.: (1) JAOAC 72, 907 (1989). (2) JAOAC