

# Detection of Sugar Syrups in Apple Juice by $\delta 2H\%$ and $\delta 13C\%$ Analysis of Hexamethylenetetramine Prepared from Fructose

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#### Introduction

Stable carbon isotope ratios are used widely for the authentication of foodstuffs, especially for the detection of added cane sugar in fruit juices, spirits and honey. The major deficiency of stable carbon isotope analysis is that it cannot be used to detect the addition of beet sugar syrups toC3 crops, as the beet plant also uses the Calvin photosynthetic pathway. However, the addition of commercial sweeteners such as beet medium invert syrup can be detected by isotope ratio mass spectrometry if the abundance of deuterium in the sugars is determined.





#### Preparation of Hexamethylenetetramine from Fructose (Figure 1).

Apple juice fructose was obtained by semi-preparative HPLC and dissolved in deionized water (5 mL). A solution containing 8 g of potassium hydrogen carbonate dissolved in 10 mL of water was added to the fructose solution and stirred. A 15% w/v periodic acid solution was then added slowly with stirring. After reaction for 45 min at 20 °C, the fructose was quantitatively degraded to formaldehyde (C1 and C6) and formic acid (C2 to C5). A total of 5 mL of a 35% w/v ammonia solution was added to the reaction mixture. After 3 h at 20 °C, the reaction of formaldehyde with ammonia produced the 6:4 tricyclic complex hexamethylenetetramine (HMT). The HMT was extracted from the reaction residue by shaking with 3 x 10 mL portions of chloroform. mL. This solution was used for isotopic analysis by 2H GC-Pyrolysis-IRMS.



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10ºC min<sup>-1</sup>

H<sub>2</sub> REF GAS

3

205°C

Retention1

1000

7e-009

7e-012

m/z 2

m/z 3

Amperes

1

145°C

### Hydrogen Isotope Analysis of Hexamethylenetetramine.(Figure 2).

The separation of the HMT from the chloroform solvent and n-hexadecane internal standard was achieved using a Agilent GC. The GC was fitted with a SGE BPX5 wide-bore capillary column (25 m x 0.53mm i.d.) of fused silica coated with 95% dimethyl-5% diphenyl polysiloxane (film thickness 0.1 (m). Helium was used as a carrier gas and injection was in splitless mode, to avoid isotopomer discrimination in the injector. The split valve was closed for 0.75 min following injection, and then purged at 100 mL/min. Injection was via an automatic sampler (Eurovector, Milan, Italy). The GC oven was temperature programmed to obtain optimum resolution between the HMT and nhexadecane internal standard.

The GC column effluent was passed into the pyrolysis interface coupled to the IRMS (PDZ-Europa, Crewe, UK). The pyrolysis interface and mass spectrometer have previously been described in detail. The manufacturer's proprietary software (PDZ-Europa, Orchid Post processor) was used for peak identification, ratio calculation, and H3+ correction. The hydrogen isotope ratios of hexamethylenetetramine were calculated with reference to the assigned value of the nhexadecane (C16) internal standard and reported in ‰ relative

to the V-SMOW.

#### **Results and discussion**

An improved procedure for determining 13C and 2H isotope ratios, using gas chromatography-isotope ratio mass spectrometry (GC-IRMS), has been developed for identifying the addition of low cost commercial sugar syrups to apple juices and related products. Isotopic techniques are commonly used to identify the addition of low cost sugars to fruit juices and are difficult to circumvent as it is not economically viable to change the isotopic ratios of the sugars. The procedure utilizes the derivative hexamethylenetetramine, which is produced through chemical transformation of a sugar degradation product and provides position-specific 13C and 2H ratios that relate to the parent sugar molecule. The new procedure has advantages over methods using nitro-sugar derivatives in terms of analysis time and sensitivity. The differences between the ä2H‰ and ä13C‰ values of the 100 authentic apple juices and beet and cane commercial sugar syrups permit their addition to be reliably detected (Figure 3).

#### Sercon Equipment References

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GC-CP interface

CF 20-20 IRMS

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