Method 1.9 - Official Methods: mixed juice sucrose

1. Rationale

This method is applicable to all factory juices and is used to determine the glucose, fructose and sucrose contents of a sample by gas chromatography.

2. Principle

The method uses gas chromatography with flame ionisation detection (FID) and an internal standard (trehalose dihydrate) to determine the glucose, fructose and sucrose contents of a sample quantitatively. Results are based on the peak area to mass ratios of an integrated chromatograph.

The sugars in a filtered juice sample are volatilized by silylation using a trimethysilyl (TMS) reagent. The TMS-ethers of the various sugars are separated from each other and from other volatile components using a low to medium polarity column.

3. Definitions

3.1 Internal standardisation

The area of a specific chromatographic peak is determined by the amount of the analyte present in the sample. However, other factors such as fluctuations in the carrier gas flow rate, column and detector temperatures may also influence the peak and will in turn affect the sensitivity response of the detector. Internal standardisation can be used to eliminate the effect of such variations by adding a known amount of a reference substance (the internal standard) to a fixed volume of the sample before injection into the column.

Standard solutions with known quantities of the analytes (glucose, fructose and sucrose) and the internal standard (trehalose) are prepared and chromatographed. The standards are prepared to bracket the expected concentration range.

3.2 Relative response factor (RRF)

A relative response factor is determined for each sugar by dividing the mass to peak area ratio of each sugar by the mass to peak area ratio of the internal standard. The RRF values should be similar for standard solutions of different concentrations.

$$RRF_s = \frac{M_s}{P_s} \times \frac{P_I}{M_I}$$

where P ≡ peak area
M ≡ mass
S ≡ sugar
I ≡ internal standard

4. Apparatus

All glassware must be boiled in a detergent (e.g. Extran, Merck), rinsed thoroughly and dried in an oven at approximately 110°C.

4.1 **Hypovials** with teflon septa: 6 cm^3

4.2 **Beakers**: 50 cm^3

4.3 **Parafilm**

4.2 **Mechanical shaker**

4.3 **Ultrasonic bath** and recirculation heater

4.4 **Schott bottle**: 250 cm^3

4.5 **Filtration apparatus**

funnel: 100 mm ϕ stemless
beakers: 3 × 250 cm^3
watch/cover glass: 100 mm ϕ

4.6 **Gas chromatograph**

Flame ionisation detection (FID)
Electronic integrator

4.6.1 Experimental conditions

Gas chromatographic conditions will vary, depending on a number of factors such as the type of chromatograph, stationary phase and loading, density of packing material and type of column (packed or capillary). Therefore, the conditions given here are only guidelines and it is essential that each analyst determine their own experimental conditions.

Oven temperature programme

Initial temp	165°C
Initial time	2 minutes (optional)
Prog rate	8°C/minute
Final temp	275°C
Final time	2 minutes (optional)
Inlet temp	275°C
FID temp	285°C
Carrier gas	N_2
Inlet pressure	100 - 200 kPa
Gas flow	0.3 - 2.0 cm^3/min
Column tubing	Fused Silica
Column dimensions	15 m × 0.53 mm id
Stationary phase	BP5
Volume injected	1 - 3 µl
Split ratio	10:1
Inlet	Glass + glass wool
Detector attenuation	64
Detector range	10^{-11}
Air flow	300 ml/min
Hydrogen flow	30 ml/min
N_2 make-up gas flow	30 ml/min

4.7 Filter paper: Whatman No. 9, Postslip medium white w/s or equivalent

4.8 Desiccator with self-indicating silica gel

4.9 Syringe: 10 µl

5. Reagents

5.1 Phosphorus pentoxide

Phosphorus pentoxide (P_2O_5) is toxic and corrosive to the eyes, skin and respiratory tract. Work in a fume cupboard while wearing gloves and safety glasses.

5.2 Glucose

Dry at approximately 40°C under vacuum over phosphorus pentoxide overnight and store in a desiccator.

5.3 Fructose

Dry at approximately 40°C under vacuum over phosphorus pentoxide overnight and store in a desiccator.

5.4 Sucrose

Dry at approximately 40°C under vacuum over phosphorus pentoxide overnight and store in a desiccator.

5.5 Trehalose dihydrate ($C_{12}H_{22}O_{11} \cdot 2H_2O$)

5.6 Pyridine - <0.1% moisture

Pyridine (C_5H_5N) is highly flammable and potentially explosive. It is irritating to the eyes, skin and reparatory tract and damaging to internal organs when inhaled or swallowed. Work in a fume cupboard while wearing gloves and safety glasses.

5.7 Hexamethyldisilazane (HMDS)

Hexamethyldisilazane ($C_6H_{19}NSi$) is flammable and irritating to the eyes, skin and respiratory tract. It will also cause severe burn when in contact with the eyes. Work in a fume cupboard while wearing gloves and safety goggles.

5.8 Trifluoroacetic acid - TFA

Trifluoroacetic acid ($C_2O_2DF_3$) is corrosive to the eyes, skin and respiratory tract and harmful if swallowed, inhaled or absorbed through the skin. Work in a fume cupboard while wearing gloves and safety glasses.

5.9 Celite 577

Celite is an inert powder and inhalation may cause asbestosis of the lungs. Wear a dust mask during use.

6. Procedure

6.1 Calibration standards

Prepare a solution of glucose and fructose by dissolving 0.200 g of glucose and fructose respectively in 9.600 g of distilled water (the total mass should be 10.000 g) in order to avoid weighing small quantities of these sugars.

Prepare three standard solutions by weighing the quantities indicated in Table 1 accurately into 6 cm^3 hypovials. Seal the vials with Parafilm and shake mechanically to dissolve.

Table 1: Calibration standards

Component	Calibration Standard		
	S1	S2	S3
Fructose/Glucose solution (g)	0.7000	0.3000	0.1500
Trehalose (g)	0.2500	0.2500	0.2500
Sucrose (g)	0.1200	0.2000	0.2600
H_2O (g)	1.2800	1.6000	1.6900

Duplicate aliquots from each of these standards must be prepared for analysis together with the samples.

6.2 Control solutions

Control solutions are a mixture of fructose, glucose and sucrose at the expected Brix of a juice sample and are treated as normal samples. Two control solutions are prepared and each is analysed in duplicate.

6.2.1 Mixed juice control samples

Accurately weigh (to 0.0001 g) the quantities indicated in Table 2 into 6 cm^3 hypovials, seal with "Parafilm" and shaken mechanically to dissolve.

Table 2: Mixed juice control solutions

Component	Control	
	C1	C2
Fructose (g)	0.2500	0.1000
Glucose (g)	0.2500	0.1000
Sucrose (g)	4.5000	6.5000
H_2O (g)	45.0000	43.3000

6.3 Samples

Weigh 0.250 g of trehalose and 2.000 g of filtered mixed juice in duplicate accurately into 6 cm^3 hypovials. Seal the vials with "Parafilm" and shake mechanically to dissolve.

6.4 Analysis

6.4.1 Sample preparation

Weigh 1 g Celite 577 powder while wearing a dust mask and gloves and add to 50 cm^3 of the sample in the 250 cm^3 Schott bottle. Mix and filter the solution through the fluted filter paper supported in the funnel which rests directly on the beaker. Seal the funnel with a watch glass to minimise evaporation. Discard the first 10 cm^3 of filtrate and collect

about 20 cm^3 of the filtrate in another clean beaker. Do not allow the filtrate to touch the bottom of the funnel or filter paper. Do not replenish the solution in the filter funnel.

Use a 10 µl syringe to transfer a 5 µl aliquot of the standard or sample solution into a labelled 2 cm^3 crimp-cap vial.

From this point onwards, all work must he carried out in a fume cupboard. Take care not to contaminate any of the chemicals. Ensure that the syringes and glassware are dry.

6.4.2 Silylation

Add 0.5 cm^3 pyridine to each vial followed by gentle swirling. Add 0.45 cm^3 HMDS to each vial and swirl gently. Add 50 µl TFA rapidly from a 100 µl syringe. Seal the vials with a Teflon-backed septum and shake vigorously. Place in an ultrasonic bath at approximately 80°C for about 10 minutes. Remove the vials and shake each in turn while still hot. At this stage samples are stable and could be kept in a refrigerator for up to one week until it is convenient to run them on the gas chromatograph.

6.4.3 Running the samples

Use injection volumes of 3 il for standards and samples. Run the three standards first, then all the samples and controls followed by the three standards at the end. Response factors from the six standards are averaged to calculate the sugar concentrations in each sample.

7. Calculations

7.1 Calibration

Use the internal standard technique on peak areas as described in 3 for calibration.

7.2 Sugar % sample

Determine the amount of each sugar in the sample as follows:

$$\% \text{ sugar} = \frac{P_s}{P_I} \times M_I \times RRF_s \times \frac{100}{M_{sple}}$$

where P ≡ peak area
M ≡ mass
S ≡ sugar
I ≡ internal standard
RRF_s ≡ relative response factor
M_{sple} ≡ mass of sample

8. Precision

The relative standard deviation (RSD) for sucrose should be less than 1% for duplicate analyses. The RSD for the monosaccharides should he less than 5% for duplicate analyses.

9. References

Anon (1986). *Proc ICUMSA,* 19: 134.

Day-Lewis CJM (1990). SMRI Technical Report No. 1579.

ICUMSA (1994). Fructose, glucose and sucrose in cane juices, syrups and molasses by gas chromatography (GC). *ICUMSA Methods Book*, GS7/4/3-22.

Schaffler KJ, and Morel du Boil PG (1984). *Sugar Technology Review* II: 94 - 184.

SMRI (2004). Determination of glucose, fructose and sucrose in cane mixed juice and molasses by gas chromatography - silylation only. *SMRI Test Methods*, TM300.