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Method of Test for Sugars in Foods

1. Scope

This method is applicable for the determination of galactose, glucose, sucrose, fructose, lactose and maltose in foods.

2. Method

After extraction, analytes are determined by high performance ion chromatography (HPIC).

2.1. Equipment

2.1.1. High performance ion chromatograph

2.1.1.1. Detector: pulsed electrochemical detector.

2.1.1.1.1. Gold working electrode.

2.1.1.1.2. Ag/AgCl reference electrode.

2.1.1.2. Column: CarboPac PA20, 3 mm × 15 cm, or an equivalent product.

2.1.1.3. Guard column: CarboPac PA20 Guard, 3 mm × 3 cm, or an equivalent product.

2.1.2. Oven: with an automatic temperature controller, capable of controlling temperature at ± 1°C.

2.1.3. Shaker.

2.1.4. Ultrasonicator.

2.1.5. Centrifuge: centrifugal force > 9000 ×g.

2.2. Chemicals

Ethanol, reagent, grade;

50% Sodium hydroxide, IC grade;

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

Galactose, glucose, sucrose, fructose, lactose and maltose, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 20 mL and 50 mL.

2.3.2. Centrifuge tube: 50 mL, PP.

2.3.3. Membrane filter: 0.22 μm, PVDF.

2.4. 50% ethanol preparation

Dilute 500 mL of ethanol with deionized water to 1000 mL.

2.5. Mobile phase preparation:

2.5.1. Solvent A, deionized water.

2.5.2. Solvent B

Dilute 10.5 mLof 50% sodium hydroxide with deionized water to 1000 mL. Filter with a membrane filter, and the filtrate as the mobile phase B.

2.6. Standard solution preparation

Transfer about 2.5 g of galactose, glucose, sucrose, fructose, lactose and maltose reference standards weighed accurately, which were predried in an oven at 70°C for at least 4 hr, to each 50-mL volume flask, dissolve and dilute to volume with 50% ethanol as the stock standard solutions. When to use, mix appropriate volume of each standard stock solution, and dilute with deionized water to 0.5~30 μg/mL as the standard solutions.

2.7. Sample solution preparation

2.7.1. Liquid sample

Remove the carbon dioxide by ultrasonication for the sample containing carbon dioxide. Transfer about 1 g of the sample weighed accurately into a centrifuge tube, add 10 mL of 50% ethanol, sonicate for 20 min, shake for 10 min, and dilute to 20 mL with 50% ethanol. Centrifuge at 9000 ×g for 10 min, take appropriate volume of the supernatant, and dilute 50 times with deionized water. Filter with a membrane filter, and take the filtrate as the sample solution.

2.7.2. Semisolid and solid sample:

Grind the sample into powder, or homogenize the sample thoroughly. Transfer about 1 g of the sample accurately weighed to a centrifuge tube. Add 10 mL of 50% ethanol, sonicate for 20 min, shake for 10 min, and dilute to 20 mL with 50% ethanol. Centrifuge at 9000 ×g for 30 min, take appropriate volume of the supernatant, and dilute 50 times with deionized water. Filter with a membrane filter, and take the filtrate as the sample solution.

2.8. Identification and quantification:

Accurately inject 10 μL of the sample solution and the standard solutions into HPIC separately, and operate according to the following conditions. Identify each sugar based on the retention time. Calculate the amount of each sugar in the sample by the following formula:

$$\text{The amount of each sugar in the sample (g/100 g)} = \frac{C \times V \times F}{M \times 10000}$$

C：the concentration of each sugar in the sample solution calculated by the

standard curve (μg/mL)

V：the final make-up volume of the sample (20 mL)

M：the weight of the sample (g)

F：dilution factor (50)

HPIC operating condition(Note)：

Detector: pulsed electrochemical detector.

Gold working electrode.

Ag/AgCl reference electrode.

Column: CarboPac PA20, 3 mm × 15 cm.

Guard column: CarboPac PA20 Guard, 3 mm × 3 cm.

Column temperature: 30°C.

Mobile phase: a gradient program of solvent A and solvent B is as follows:

Time (min)	A (%)	B (%)
0 → 15	97 → 90	3 → 10
15 → 22	90 → 0	10 → 100
22 → 29	0 → 0	100 → 100
29 → 29.1	0 → 97	100 → 3
29.1 → 35	97 → 97	3 → 3

Flow rate: 0.5 mL/min.

Injection volume: 10 μL.

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

Remark

1. Limits of quantification (LOQs) are 0.05 g/100 g for galactose, glucose, sucrose, fructose, lactose and maltose.
2. Further validation should be performed when interfering compounds appear in samples.

References

1. AOAC. 1995. Carbohydrates in soluble (instant) coffee. AOAC Official Method 995.13.
2. AOAC. 2003. Determination of trace glucose and fructose in raw cane sugar. AOAC Official Method 2000.17.

Reference chromatogram

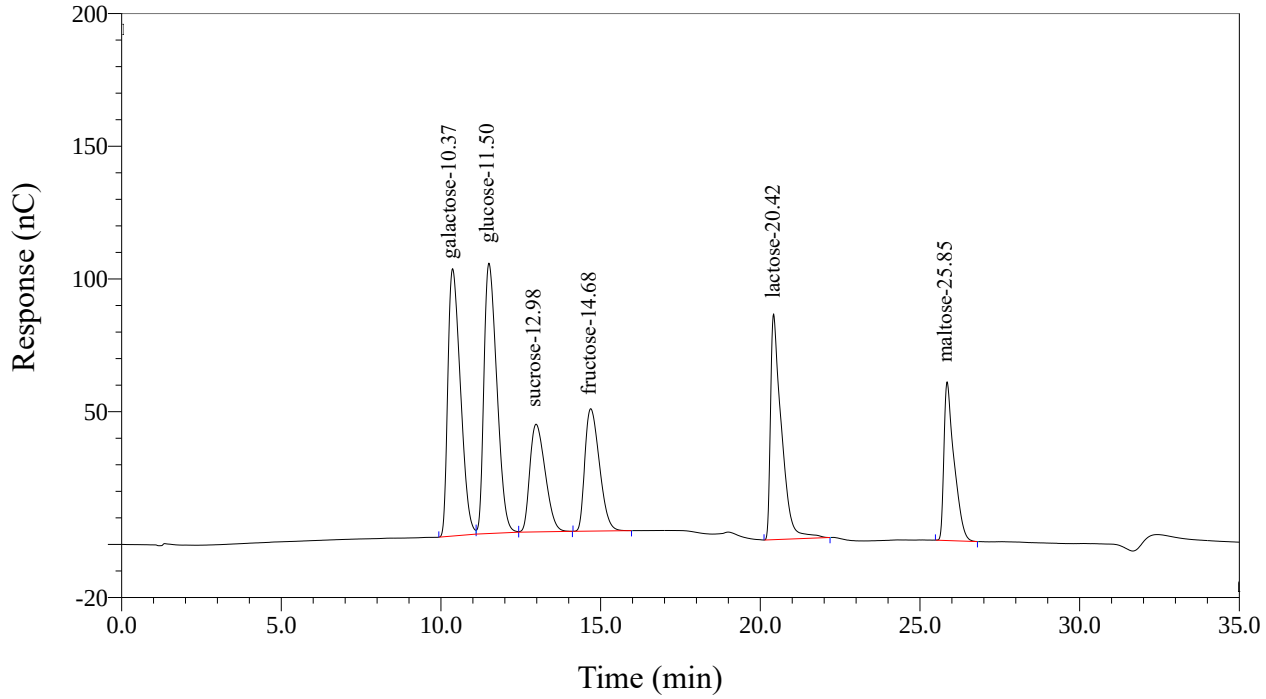

Figure. The HPIC chromatogram of galactose, glucose, sucrose, fructose, lactose and maltose standards.