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National food safety standard - Determination of fructose, glucose, sucrose, maltose and lactose in foods

食品安全国家标准　食品中果糖、葡萄糖、蔗糖、麦芽糖、乳糖的测定

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National food safety standard

Determination of fructose, glucose, sucrose, maltose and lactose in foods

1 Scope

This Standard specifies the methods for the determination of fructose, glucose, sucrose, maltose and lactose in foods.

Method 1 - High performance liquid chromatography applies to the determination of fructose, glucose, sucrose, maltose and lactose in grains and grain products, milk and dairy products, fruits, vegetables and fruit and vegetable products, sweeteners, candies, beverages and infant foods.

Method 2 - Ion chromatography applies to the determination of fructose, glucose, sucrose, maltose, and lactose in foods.

Method 3 - Acid hydrolysis-Rhein-Eynon method applies to the determination of sucrose in foods.

Method 4 - Rhine-Enon method applies to the determination of lactose in infant foods and dairy products.

Method 1 - High performance liquid chromatography

2 Principle

The fructose, glucose, sucrose, maltose and lactose in the sample are extracted, and then separated by high performance liquid chromatography column, detected by a differential refractive index detector or an evaporative light scattering detector, and quantified by the external standard method.

3 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, and the water is grade-1 water specified by GB/T 6682.

3.1 Reagents

3.1.1 Acetonitrile (C_2H_3N): chromatographic purity.

3.1.2 Zinc acetate [$Zn(CH_3COO)_2 \cdot 2H_2O$].

3.1.3 Potassium ferrocyanide [$K4Fe(CN)_6 \cdot 3H_2O$].

3.1.4 Glacial acetic acid (CH_3COOH).

3.2 Preparation of reagents

3.2.1 Zinc acetate solution (1 mol/L): Weigh 21.9 g of zinc acetate; add 3 mL of glacial acetic acid; dissolve in water and dilute to 100 mL; mix well.

3.2.2 Potassium ferrocyanide solution (0.25 mol/L): Weigh 10.6 g of potassium ferrocyanide; dissolve in water and dilute to 100 mL; mix well.

3.3 Standards

3.3.1 Fructose ($C_6H_{12}O_6$, CAS number: 57-48-7): purity ≥99%, or standard material certified by the country and awarded a standard material certificate.

3.3.2 Glucose ($C_6H_{12}O_6$, CAS number: 50-99-7): purity ≥99%, or standard material certified by the country and awarded a standard material certificate.

3.3.3 Sucrose ($C_{12}H_{22}O_{11}$, CAS number: 57-50-1): purity ≥99%, or standard material certified by the country and awarded a standard material certificate.

3.3.4 Maltose ($C_{12}H_{22}O_{11}$, CAS number: 69-79-4): purity ≥99%, or standard material certified by the country and awarded a standard material certificate.

3.3.5 Lactose ($C_{12}H_{22}O_{11}$, CAS number: 63-42-3): purity ≥99%, or standard material certified by the country and awarded a standard material certificate.

3.4 Preparation of standard solutions

3.4.1 Mixed standard stock solution (20.0 mg/mL): Respectively weigh 1 g (accurate to 0.001 g) each of fructose dried at 90 °C ± 2 °C for 2 h and glucose, sucrose, maltose and lactose dried at 96 °C ± 2 °C for 2 h; dissolve in water and then transfer to the 50 mL volumetric flasks; add 2.5 mL of acetonitrile; use water to adjust the volume to the mark. Store at 0 °C ~ 4 °C and seal, with a shelf life of 3 months.

3.4.2 Mixed standard working solution: Take 0.100 mL, 1.00 mL, 2.00 mL, 3.00 mL and 5.00 mL of mixed standard stock solution (20.0 mg/mL) into the 10.0 mL volumetric flask; use water to dilute to the mark, to prepare the mixed standard working solutions of fructose, glucose, sucrose, maltose and lactose with mass concentrations of 0.200 mg/mL, 2.00 mg/mL, 4.00 mg/mL, 6.00 mg/mL and 10.0 mg/mL. The concentration of the mixed standard working solution can be appropriately adjusted according to the concentration of the actual sample solution. Prepare when necessary.

3.5 Materials

Weigh 50 g (accurate to 0.01 g) of the mixed sample in an evaporating dish; stir gently on a water bath to remove gas and alcohol; transfer to a 100 mL volumetric flask after cooling; slowly add 5 mL of zinc acetate solution and 5 mL of potassium ferrocyanide solution; use water to adjust the volume to the mark; mix well; let stand.

5.1.2.3 Syrup and honey samples

Weigh 1 g ~ 2 g (accurate to 0.001 g) of the mixed sample into a 100 mL colorimetric tube; add about 50 mL of water; vortex and mix until fully dissolved; transfer to a 100 mL volumetric flask; use water to fix the volume to the mark; mix well; let stand.

5.1.2.4 Other samples

Weigh 1 g ~ 10 g (accurate to 0.001 g) of the crushed or mixed sample (10 g when the target sugar content is ≤5%; 5 g when the content is 5% ~ 10%; 2 g when the content is 10% ~ 40%; 1 g when the content is ≥40%) into a 100 mL colorimetric tube; add about 50 mL of water; then, slowly add 5 mL of zinc acetate solution and 5 mL of potassium ferrocyanide solution; vortex and mix, and sonicate for 30 minutes; transfer to a 100 mL volumetric flask; use water to adjust the volume to the mark; mix well; let stand.

5.1.3 Purification

For the above sample extract solution, use a filter paper to filter (discard the primary filtrate) or centrifuge to obtain the supernatant; then, use a 0.45 μm water-based membrane syringe to filter into a sample bottle for analysis by a high-performance liquid chromatograph.

5.2 Apparatus reference conditions

Apparatus reference conditions are as below:

a) Chromatographic column: Amino chromatographic column (4.6 mm × 250 mm, particle size 5 μm, aminosilane bonded silica gel as filler), or one with equivalent performance;

b) Mobile phase: acetonitrile + water = 70 + 30 (volume ratio);

c) Flow velocity: 1.0 mL/min;

d) Column temperature: 40 ℃;

e) Injection volume: 10 μL;

f) Differential refractive index detector conditions: temperature 40 °C;

g) Evaporative light scattering detector conditions: drift tube temperature 80 °C ~ 90 °C; nitrogen flow rate 2.5 L/min.

5.3 Preparation of the standard curve

Inject the mixed standard working solution into the high-performance liquid chromatograph in order from low to high concentration, and measure the corresponding peak areas or peak heights of fructose, glucose, sucrose, maltose and lactose. For the differential refractive index detector, use the concentration of the standard working fluid as the abscissa and the peak area or peak height as the ordinate to draw a standard curve; for the evaporative light scattering detector, use the power function of the concentration of the standard working solution as the abscissa and the power function of the peak area or peak height as the ordinate to draw a standard curve. Refer to Appendix A for HPLC chromatograms of standard solutions of fructose, glucose, sucrose, maltose and lactose.

5.4 Determination of sample solution

Inject the sample solution into the high-performance liquid chromatograph; qualitatively record the peak area or peak height of the target substance based on the retention time; obtain the concentrations of fructose, glucose, sucrose, maltose and lactose in the sample solution based on the standard curve.

5.5 Blank test

Except that no sample is added, proceed according to the above steps.

6 Expression of analysis results

Calculate the contents of fructose, glucose, sucrose, maltose and lactose in the sample according to Formula (1).

$$X = \frac{(\rho - \rho_0) \times V \times f}{m \times 1\,000} \times 100 \qquad \cdots\cdots(1)$$

Where:

X – the content of fructose, glucose, sucrose, maltose and lactose in the sample, in grams per hundred grams (g/100 g);

ρ – the mass concentration of fructose, glucose, sucrose, maltose and lactose in the sample solution obtained according to the standard curve, in milligrams per milliliter (mg/mL);

ρ_0 – the mass concentration of fructose, glucose, sucrose, maltose and lactose in the blank obtained according to the standard curve, in milligrams per milliliter (mg/mL);

V – constant volume, in milliliters (mL);

f – dilution factor;

10.2.2 Acetic acid solution (3%, volume fraction): Measure 3 mL of glacial acetic acid; use water to dilute to 100 mL; mix well.

10.3 Standards

10.3.1 Fructose ($C_6H_{12}O_6$, CAS number: 57-48-7): purity ≥99%, or standard material certified by the country and awarded a standard material certificate.

10.3.2 Glucose ($C_6H_{12}O_6$, CAS number: 50-99-7): purity ≥99%, or standard material certified by the country and awarded a standard material certificate.

10.3.3 Sucrose ($C_{12}H_{22}O_{11}$, CAS number: 57-50-1): purity ≥99%, or standard material certified by the country and awarded a standard material certificate.

10.3.4 Maltose ($C_{12}H_{22}O_{11}$, CAS number: 69-79-4): purity ≥99%, or standard material certified by the country and awarded a standard material certificate.

10.3.5 Lactose ($C_{12}H_{22}O_{11}$, CAS number: 63-42-3): purity ≥99%, or standard material certified by the country and awarded a standard material certificate.

10.4 Preparation of standard solution

10.4.1 Mixed standard stock solution (10.0 mg/mL): Weigh 1 g (accurate to 0.001 g) each of fructose dried at 90 °C ± 2 °C for 2 h and glucose, sucrose, maltose and lactose dried at 96 °C ± 2 °C for 2 h; add water to dissolve and transfer to a 100 mL volumetric flask; add 2 mL of acetic acid solution; use water to dilute to the mark. Store at 0 °C ~ 4 °C and seal, with a shelf life of 3 months.

10.4.2 Mixed standard intermediate solution (100 mg/L): Pipette 1.00 mL of fructose, glucose, sucrose, maltose and lactose mixed standard stock solution (10.0 mg/mL) into the 100 mL volumetric flask; use water to dilute to the mark. Store at 0 °C ~ 4 °C and seal, with a shelf life of 1 months.

10.4.3 Mixed standard working solution: Respectively take 0.250 mL, 0.500 mL, 1.00 mL, 2.00 mL and 2.50 mL of the mixed standard intermediate solution (100 mg/L) into the 10 mL volumetric flasks; use water to dilute to the mark, to prepare the mixed standard working solutions of fructose, glucose, sucrose, maltose and lactose with mass concentrations of 2.50 mg/L, 5.00 mg/L, 10.0 mg/L, 20.0 mg/L and 25.0 mg/L. The concentration of the mixed standard working solution can be appropriately adjusted according to the concentration of the actual sample solution. Prepare when necessary.

10.5 Materials

10.5.1 0.45 μm water-based membrane syringe filter (except cellulose membrane).

10.5.2 Purification column: C_{18} solid-phase extraction cartridge (1.0 mL) or one with equivalent performance.

10.5.3 Syringe.

11 Instruments and apparatuses

11.1 Ion chromatograph: equipped with pulse ampere detector.

11.2 Sample crushing equipment: high-speed crusher.

11.3 Ultrasonic cleaner.

11.4 Analytical balance: sensitivity 1 mg.

11.5 Vortex mixer.

11.6 Centrifuge: speed ≥ 4 000 r/min.

11.7 Constant-temperature drying oven.

11.8 Constant-temperature water bath device.

12 Test steps

12.1 Sample pretreatment

12.1.1 Sample preparation

Take an appropriate amount of representative sample; for drinks and other liquid homogeneous samples, shake directly; for non-uniform samples, homogenize or crush evenly; for frozen drinks, melt at room temperature, stir thoroughly and, if necessary, heat and stir in a water batch at 30 °C ~ 40 °C; for sauces, grind or homogeneously mix; for chocolate, heat and melt in a water bath at 50 °C ~ 60 °C, and stir thoroughly while it is hot.

12.1.2 Sample extraction

12.1.2.1 Insoluble samples such as gum-based candies and chocolates

Accurately weigh 2 g (accurate to 0.001 g) of the sample into a 100 mL colorimetric tube; add about 50 mL of 50 °C ~ 60 °C hot water; vortex or stir, until the sample is fully dissolved; add 2 mL of acetic acid solution; vortex to mix, and sonicate for 30 min; transfer to a 100 mL volumetric flask and use water to adjust the volume to the mark; mix well; let stand for 20 minutes.

12.1.2.2 Syrup and honey samples

Weigh 2 g (accurate to 0.001 g) of the mixed sample into a 100 mL colorimetric tube; add about 50 mL of water; vortex and mix until fully dissolved; transfer to a 100 mL

volumetric flask; use water to adjust the volume to the mark; mix well; let stand for 20 minutes.

12.1.2.3 Samples containing gas and alcohol

Accurately weigh 10 g of the sample (accurate to 0.001 g) in an evaporating dish; stir gently on a water bath to remove gas and alcohol; after cooling, transfer it to a 100 mL volumetric flask with water; add 2 mL of acetic acid solution; use water to adjust the volume to the mark; mix well; let stand for 20 minutes.

12.1.2.4 Other samples

Weigh 2 g (accurate to 0.001 g) of solid sample and 5 g ~ 10 g (accurate to 0.001 g) of semi-solid or liquid sample into a 100 mL colorimetric tube; add about 50 mL of water; vortex to mix; then, add 2 mL of acetic acid solution; after mixing, sonicate for 30 minutes; transfer to a 100 mL volumetric flask and use water to dilute to the mark; mix well; let stand for 20 minutes.

12.1.3 Sample purification

The sample extraction solution can be diluted by an appropriate multiple according to the target sugar content in the sample. When detecting lactose in infant formula milk powder, dilute it 1 000 times and then purify it; when detecting high sugar content in honey and candy, dilute it 500 times and then purify it.

After filtering or centrifuging the above sample extraction solution or dilution solution to obtain the supernatant, pass it through a 0.45 μm aqueous membrane syringe filter and a C_{18} solid phase extraction cartridge (1.0 mL) in sequence; discard the first 3 mL; collect subsequent eluates for testing.

Before use, pass 10 mL of methanol and 15 mL of water in sequence through the C_{18} solid-phase extraction cartridge (1.0 mL); let stand to activate for 30 min.

12.2 Apparatus reference conditions

Apparatus reference conditions are as below:

a) Chromatographic column: anion exchange column (4 mm × 250 mm, particle size 10 μm, quaternary ammonium salt as functional group, polystyrene/divinylbenzene polymer resin as filler) (with guard column 4 mm × 50 mm), or one with equivalent performance;

b) Flow velocity: 1.0 mL/min;

c) Injection volume: 10 μL;

d) Pulse ampere detector: Au working electrode; see Table 1 for sugar detection waveform reference conditions;

16 Principle

After the protein is removed from the sample, the sucrose is hydrolyzed by hydrochloric acid and converted into reducing sugar, and is measured as reducing sugar. The difference before and after hydrolysis multiplied by the corresponding coefficient is the sucrose content. Raffinose, stachyose, galactooligosaccharides, fructans, polydextrose and resistant dextrin will interfere with the determination of sucrose.

17 Reagents and solutions

Unless otherwise specified, all the reagents in this method are analytical reagents, and the water is grade-3 water specified by GB/T 6682.

17.1 Reagents

17.1.1 Zinc acetate [$Zn(CH_3COO)_2 \cdot 2H_2O$].

17.1.2 Potassium ferrocyanide [$K_4Fe(CN)_6 \cdot 3H_2O$].

17.1.3 Hydrochloric acid (HCl).

17.1.4 Sodium hydroxide (NaOH).

17.1.5 Methyl red ($C_{15}H_{15}N_3O_2$): indicator agent.

17.1.6 Methylene blue ($C_{16}H_{18}ClN_3S \cdot 3H_2O$): indicator agent.

17.1.7 Copper sulfate ($CuSO_4 \cdot 5H_2O$).

17.1.8 Potassium sodium tartrate ($C_4H_4O_6KNa \cdot 4H_2O$).

17.1.9 Glacial acetic acid (CH_3COOH).

17.1.10 Ethanol (C_2H_5OH): 95%.

17.2 Preparation of reagents

17.2.1 Zinc acetate solution (1 mol/L): Weigh 21.9 g of zinc acetate; add 3 mL of glacial acetic acid; dissolve in water and dilute to 100 mL; mix well.

17.2.2 Potassium ferrocyanide solution (0.25 mol/L): Weigh 10.6 g of potassium ferrocyanide; dissolve in water and dilute to 100 mL; mix well.

17.2.3 Hydrochloric acid solution (50%, volume fraction): Weigh 50 mL of hydrochloric acid; slowly add 50 mL of water; let it cool; mix well.

17.2.4 Sodium hydroxide solution (40 g/L): Weigh 4.0 g of sodium hydroxide; add water to dissolve; cool; use water to dilute to 100 mL; mix well.

17.2.5 Methyl red indicator solution (1 g/L): Weigh 0.1 g of methyl red; use 95% ethanol to dissolve and dilute to 100 mL; mix well.

17.2.6 Sodium hydroxide solution (200 g/L): Weigh 20.0 g of sodium hydroxide; add water to dissolve; cool; use water to dilute to 100 mL; mix well.

17.2.7 Alkaline cupric tartrate solution A: Weigh 15.0 g of copper sulfate and 0.05 g of methylene blue; dissolve in water and dilute to 1 000 mL; mix well.

17.2.8 Alkaline cupric tartrate solution B: Weigh 50.0 g of sodium potassium tartrate and 75.0 g of sodium hydroxide; dissolve in water; then, add 4.0 g of potassium ferrocyanide; after complete dissolution, use water to dilute to 1 000 mL; mix well; store in a glass bottle with rubber stopper.

17.3 Standard

Glucose ($C_6H_{12}O_6$, CAS number: 50-99-7): purity ≥99%, or standard material certified by the country and awarded a standard material certificate.

17.4 Preparation of standard solutions

Glucose standard solution (1.00 mg/mL): Weigh 1 g (accurate to 0.001 g) of glucose that has been dried in an oven at 96 °C ± 2 °C for 2 hours; add water to dissolve; transfer to a 1 000 mL volumetric flask; add 5 mL of hydrochloric acid; use water to adjust the volume to the mark. Store in a sealed container at 0 °C ~ 4 °C.

18 Instruments and apparatuses

18.1 Analytical balance: the sensitivity is 1 mg and 10 mg.

18.2 Constant-temperature water bath device.

18.3 Adjustable-temperature electric stove.

18.4 Acid burette: 25 mL.

18.5 Sample crushing equipment: high-speed crusher.

18.6 Constant-temperature drying oven.

19 Analysis steps

19.1 Sample pretreatment

19.2 Acid hydrolysis

Take 2 portions of the sample treatment solution, 50.0 mL each, and place them in the 100 mL volumetric flasks, respectively.

19.2.1 Before transformation: Use water to dilute 1 portion to the mark and mix well.

19.2.2 After transformation: Add 5 mL of hydrochloric acid solution to 1 portion; heat in a water bath at 68 °C ~ 70 °C for 15 minutes; add 2 drops of methyl red indicator solution after cooling; use sodium hydroxide solution (200 g/L) to neutralize to neutrality; use water to adjust the volume to the mark; mix well.

19.3 Calibration of alkaline cupric tartrate solution

Take 5.0 mL of alkaline cupric tartrate solution A and 5.0 mL of alkaline cupric tartrate solution B into a 150 mL Erlenmeyer flask; add 10 mL of water; add 2 ~ 4 glass beads; add about 9 mL of glucose standard solution from the burette; control the heating-to-boiling process within 2 minutes; add glucose standard solution dropwise at a rate of 1 drop/2 seconds while it is hot, until the blue color of the solution just fades away as the end point; record the total volume of glucose standard solution consumed; operate 3 portions in parallel at the same time, and take the average value. Calculate the mass A (mg) of glucose equivalent to each 10 mL of alkaline cupric tartrate solution (5 mL each of alkaline tartaric acid solutions A and B).

Note: 4 mL ~ 20 mL of alkaline cupric tartrate solution (half-and-half solutions A and B) can also be calibrated according to the above method to adapt to the concentration changes of reducing sugars in the sample.

19.4 Determination of sample solution

19.4.1 Predictive titration: Take 5.0 mL of alkaline cupric tartrate solution A and 5.0 mL of alkaline cupric tartrate solution B into a 150 mL Erlenmeyer flask; add 10 mL of distilled water; add 2 ~ 4 glass beads; heat on an electric furnace; let it boil within 2 minutes; maintain the boiling state for 15 seconds; drop in the sample solution before transformation (19.2.1) or the sample solution after transformation (19.2.2) until the blue color of the solution just fades away, which is the end point; read the volume of the sample solution used.

19.4.2 Accurate titration: Take 5.0 mL of alkaline cupric tartrate solution A and 5.0 mL of alkaline cupric tartrate solution B into a 150 mL Erlenmeyer flask; add 10 mL of distilled water; add 2 ~ 4 glass beads; release the sample solution 1 mL less than the predicted volume of the predictive titration (19.4.1) from the burette; place it on the electric stove and let it boil within 2 minutes; maintain the boiling state for 2 minutes; slowly add the sample solution at a rate of 1 drop/2 seconds until the blue color of the solution just fades as the end point; record the volume (V) of the sample solution consumed.

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