



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 5009.255-2016

**National food safety standard - Determination of fructan in
foods**

食品安全国家标准 食品中果聚糖的测定

(English Translation)

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Table of Contents

National food safety standard...	3
1 Scope...	3
2 Principles...	3
3 Reagents and materials...	3
4 Instruments and equipment...	6
5 Analytical procedures...	6
6 Expression of analysis results...	9
7 Precision...	10
8 Others...	10

1 Scope

This standard specifies the determination of fructan content in food by ion chromatography.

This standard applies to the determination of oligofructose, polyfructose or inulin content separately added in milk and dairy products, infants and young children's formula, infants and young children cereals, solid beverages and formulated wines.

2 Principles

The sample is subjected to digestion by hot water, AND the sucrose in the sample solution is hydrolyzed to glucose and fructose by sucrase. Glucose and fructose are reduced to corresponding sugar alcohol by sodium borohydride, AND the excess sodium borohydride is neutralized by acetic acid.

The fructan in the sample solution are hydrolyzed into fructose and glucose by the fructanase.

3.1 Reagents

Unless otherwise stated, the reagents used in this method are of analytical grade AND water is level I water as specified in GB/T 6682.

3.1.1

Sodium hydroxide (NaOH).

3.1.2

Maleic acid ($C_4H_4O_4$).

3.1.9

Anhydrous sodium acetate (CH_3COONa). purity $\geq 99.0\%$.

3.1.10

Nitrogen (N_2). purity $\geq 99.9\%$.

3.2.1 Sodium hydroxide solution (1 mol/L). WEIGH 40 g of sodium hydroxide

(accurate to 0.01 g); DISSOLVE it in water and DILUTE it to 1000 mL; AND it can be preserved for 2 months at room temperature.

3.2.2 Sodium maleate buffer solution (100 mmol/L, pH 6.5). WEIGH 1.16 g of

maleic acid (accurate to 0.01 g); PLACE it in a 150 mL beaker; ADD about 70 mL of water to dissolve it; USE 1 mol/L sodium hydroxide solution to adjust the pH to 6.5; USE water to dilute it to 100 mL; PRESERVE it at 4 °C, AND it can be stored for 3 months.

3.2.7 Sodium acetate solution (200 mmol/L). WEIGH 1.36 g of sodium

trihydrate (accurate to 0.01 g); USE water to dissolve and dilute it to 50 mL;

PRESERVE it at 4 °C; AND it can be stored for 2 months.

3.2.8 Sodium acetate buffer solution (pH 4.5). PIPETTE 14 mL of acetic acid

solution and 11 mL of sodium acetate solution; MIX it; USE water to dilute it to 50 mL; PREPARE it before use.

3.2.9 Fructanase solution (455 U/mL). DISSOLVE the fructanase (activity

10000 U) in 22 mL of sodium acetate buffer solution; DISPENSE it into 2 mL centrifuge tube; PRESERVE it at -20 °C and it can be stored for 6 months. It shall determine the enzyme activity before use.

3.3 Standard substance

Fructose standard substance ($C_6H_{12}O_6$). purity $\geq 99.0\%$.

3.4.1 Fructose stock solution (2000 mg/L). accurately WEIGH 0.05 g (accurate

to 0.1 mg) of fructose standard substance which had been dried to constant weight at 80 °C into a 50 mL beaker; ADD about 10 mL of hot water; until the fructose is dissolved, COOL it to room temperature; USE water to dilute it into a 25 mL volumetric flask; SHAKE it

uniformly; PRESERVE it at 4 °C and it can be stored for 1 month.

3.4.2 Fructose intermediate solution (80.0 mg/L). PIPETTE 1 mL of fructose
stock solution; USE water to dilute it into a 25 mL volumetric flask; PREPARE it before use.

3.5.1 Purification column. reverse phase solid phase extraction column, the
filler is styrene divinyl benzene, 2.5 mL.

3.5.2 Microporous membrane. water phase, 0.22 µm.

4.1 Ion chromatograph. equipped with ternary and above gradient pump, pulse
amperometric detector, and Au working electrode.

4.5 Constant temperature water bath shaker. temperature control accuracy ±
1 °C.

4.6 Centrifuge. speed ≥ 3000 r/min.

5.1.1.1 Solid sample. SPLIT it to about 200 g by "quartering" method; USE
crusher to crush it; MIX it uniformly; PREPARE for use.

5.1.2 Extraction

Accurately WEIGH 1 g ~ 5 g (accurate to 0.001 g, containing at least 5 mg of fructan) of
sample in a 150 mL conical flask; ADD about 50 mL of 80 °C ± 1 °C hot water; PLACE it in
a 80 °C ± 1 °C constant temperature water bath shaker;

SHAKE it at 150 r/min for 15 min; TAKE it out;

5.2.1 Instrument reference conditions 1

5.2.1.1

Columns. High-capacity anion exchange columns that are compatible with gradient elution.

5.2.2.2 Eluent. A. sodium hydroxide solution (90 mmol/L); B. sodium hydroxide

(150 mmol/L) and sodium acetate (500 mmol/L) mixed solution. The gradient elution
conditions are as shown in Table 3.

5.2.2.4 Column temperature. 32 °C.

5.2.2.5 Injection volume. 20 µL.

5.3 Production of standard curve

INJECT the standard curve working solution (from high to low) in turn into the ion chromatograph; DETERMINE the corresponding response value (peak area); USE the mass concentration of fructose in the standard working solution as the abscissa AND the response value (peak area) as the ordinate, DRAW the standard curve. The standard solution chromatogram is as shown in

Appendix A.

5.4 Determination of sample solution

INJECT the sample solution into the ion chromatograph; RECORD the chromatogram. MAKE qualitative based on the retention time; RECORD the peak area;

6 Expression of analysis results

The content of fructans in the sample is calculated in accordance with the equation (1), equation (2) and equation (3).

7 Precision

The absolute difference between the two independent determinations obtained under repeatability conditions shall not exceed 10% of the arithmetic mean.

8 Others

When the sample amount is 5 g, the detection limit is 0.4 g/kg AND the quantification limit is 2.0 g/kg.

Appendix A

Fructose standard solution and milk powder sample chromatogram

A.1 Fructose standard solution chromatogram (reference condition 1)

The fructose standard solution chromatogram (reference condition 1) is as shown in Figure A.1.

A.2 Milk powder sample chromatogram (reference condition 1)

The milk powder sample chromatogram (reference condition 1) is as shown in Figure A.2.

Appendix B