



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

GB 5009.276-2016

**National food safety standard - Determination of Glucono
Delta-Lactone in Foods**

食品安全国家标准 食品中葡萄糖酸- δ -内酯的测定

(English Translation)

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1 Application Scope

This Standard specifies methods for the determination of glucono delta-lactone in foods.

Method 1 of this standard is applicable to the determination of glucono delta-lactone content in meat, meat products, soy products and beverages; method 2 of this standard is applicable to the determination of glucono delta-lactone content in foods.

Method 1 -- Spectrophotometry

2 Principle

Use boiling water to extract glucono delta-lactone in the sample; filter the supernatant; under the action of potassium hydroxide, glucono delta-lactone is converted to gluconate. Under the action of gluconokinase and 6-phosphogluconate dehydrogenase, gluconate produces ribulose 5-phosphate and reduces nicotinamide adenine dinucleotide phosphate (NADP). Use a spectrometer to measure the absorbance of the reduced nicotinamide

adenine dinucleotide phosphate; then calculate the content of glucono delta-lactone.

3 Reagents and Materials

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

3.1 Reagents

3.1.1

Absolute ethanol (C₂H₆O).

6-gluconate gluconate

Gluconate adenosine triphosphate

6-phosphogluconate ribulose 5-phosphate adenosine diphosphate 6-phosphate gluconate
Gluconokinase

4.2 Analytical balance: the sensitivity is 1 mg and 0.1 mg.

4.3 Homogenizer.

5.1.1 Solid samples such as meat, meat products and soy products

Take no less than 200 g of representative samples; then use homogenizer to make them into homogenate. Analyze the finished sample as soon as possible, or seal and freeze the sample if it is not analyzed immediately. The stored sample should be remixed when activated.

5.1.2 Liquid samples such as beverage

Shake up and take samples directly.

5.2 Extraction

Meat products: weigh 2 g ~ 10 g of sample (accurate to 0.001g) into a 50 mL beaker; add 10 mL of petroleum ether to wash; use glass rod to stir evenly; let stand for 15 min; then use dry filter paper to filter; repeat washing three times. Then use 10 mL of absolute ethanol to wash for three times. Then, transfer the residue on the filter paper to the original small beaker; add 30 mL of water to boil. After cooling, use potassium hydroxide solution (2 mol/L) to adjust the solution pH to 10; use water to fix-volume to a 100 mL volumetric flask. Filter; put the filtrate as standby for the machine.

Soy products: weigh 2 g ~ 10 g of sample (accurate to 0.001 g) into a 50 mL beaker; add 30 mL of water to extract; after boiling and cooling, use potassium hydroxide solution (2

mol/L) to adjust the solution pH to 10.0; use water to fix-volume to a 100 mL volumetric flask. Filter; put the filtrate as standby for the machine.

Beverage: filter, accurately draw 30 mL of filtrate; use potassium hydroxide solution (2 mol/L) to adjust solution pH to 10; use water to fix-volume to a 100 mL volumetric flask; put as standby for the machine.

5.3 Determination

Take two 1 cm cuvettes; one of them is test cuvette and the other one is blank cuvette.

Add 2.50 mL of buffer (3.2.2), 0.1 mL of NADP solution (10 mg/mL) and 0.1 mL of ATP solution (50 mg/mL) respectively and orderly; cover and mix; add 0.2 mL of extract to the test cuvette and 0.2 mL of water to the blank cuvette respectively; then cover and mix. Then add 0.05 mL of 6-PGDH solution (2.0 mg/mL); cover and mix; then put aside for 5 min; with reference to the blank, measure the absorbance at the wavelength of m -- sample quality; the unit is g.

The calculation result shall keep three significant figures.

7 Precision

The absolute difference of two independent test results under repeatability cannot exceed 10% of the arithmetic mean value.

8 Others

When the sample weight is 10 g, the method detection-limit is 0.007 5%; the quantitation-limit is 0.025%.

Method 2 -- High Performance Liquid Chromatography

9 Principle

Glucono delta-lactone slowly hydrolyzes in water to form gluconic acid and a small amount of glucono-gamma-lactone and achieves hydrolysis equilibrium. After the sample is boiled, extracted, fix-volume and filtered, use high performance liquid chromatography to separate it. By comparing the peak area of gluconic acid in the test solution with the peak area of glucono delta-lactone standard-hydrolysis into gluconic acid, use external standard method to fix-volume and calculate the content of glucono delta-lactone in the sample.

10 Reagents and Materials

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

10.1 Reagents

10.1.1

Monopotassium phosphate (KH_2PO_4).

10.1.2

Phosphoric acid (H_3PO_4).

10.2 Reagent preparation

Monopotassium phosphate-phosphoric acid buffer: weigh 1.36 g of monopotassium phosphate and 0.5 mL of phosphoric acid; use water to dissolve them; then fix-volume

12.3 Apparatus reference conditions

12.3.1

Chromatographic column

Organic acid column with a column length of 4.6 mm, an inner diameter of 250 mm and a film thickness of 5 μm , or other silica gel bonding column with equivalent separation effect.

12.3.2

Column temperature

30°C.

12.3.3

Differential detector detection cell temperature

35°C.

12.3.4

Mobile phase

Monopotassium phosphate-phosphoric acid buffer.

12.3.5

Flow velocity

0.8 mL/min.

12.3.6

Injection volume

1 μL .

12.4 Preparation of the standard curve

Respectively inject standard series working solution into the high performance liquid chromatography to test the corresponding peak area of gluconic acid; use the concentration