GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.270-2023

National Food Safety Standard - Determination of Inositol in Foods

食品安全国家标准 食品中肌醇的测定

Issued on: September 6, 2023 **Implemented on: March 6, 2024**

Issued by: National Health Commission of the People's Republic of China;

State Administration for Market Regulation.

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National Food Safety Standard - Determination of Inositol in Foods

1 Scope

This Standard specifies the methods for the determination of inositol (myo-inositol) in foods.

Method 1 - gas chromatography is applicable to the determination of inositol in infant formulas, formulas for special medical purposes, milk and dairy products, and beverages.

Method 2 - microbiological method is applicable to the determination of inositol in foods.

Method I - Gas Chromatography

2 Principle

The inositol in the specimen is extracted with water and precipitated with ethanol. After the supernatant is centrifuged and dried, it is derivatized with a silanization reagent. The derivative is extracted with n-hexane, separated by gas chromatography and detected by a hydrogen flame ionization detector. Adopt the external standard method for quantitative determination.

3 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-1 water specified in GB/T 6682.

3.1 Reagents

3.1.1 Absolute ethanol (C_2H_6O).

3.1.2 95% ethanol (C_2H_6O).

3.1.3 Acetonitrile (C_2H_3N).

3.1.4 n-Hexane (C_6H_{14}).

3.1.5 Trimethylchlorosilane (C_3H_9ClSi).

3.1.6 Hexamethyldisilazane ($C_6H_{19}NSi_2$).

3.1.7 *N*, *N*-dimethylformamide (C_3H_7NO).

3.1.8 Anhydrous sodium sulfate (Na_2SO_4).

3.2 Preparation of Reagents

3.2.1 70% ethanol: measure-take 700 mL of absolute ethanol, use water to reach a constant volume of 1,000 mL, and evenly mix it.

3.2.2 Silanization reagent: respectively draw-take trimethylchlorosilane, hexamethyldisilazane and *N*, *N*-dimethylformamide, mix them in a volume ratio of 1 : 2 : 8, and conduct ultrasonic mixing. Prepare it right before use.

NOTE: if the silanization reagent appears white and turbid, it needs to be prepared again.

3.3 Reference Material

Inositol reference material ($C_6H_{12}O_6$, CAS: 87-89-8): purity ≥ 99%, or a standard substance certified by the state and awarded a reference material certificate.

3.4 Preparation of Standard Solutions

3.4.1 Inositol standard stock solution (1.00 mg/mL): weigh-take 100 mg (accurate to 0.1 mg) of inositol reference material that has been dried at 105 °C ± 2 °C to a constant mass, use 25 mL of water to dissolve it, and use 95% ethanol to reach a constant volume of 100 mL, and evenly mix it. Store it at 2 °C ~ 8 °C. It shall remain valid for 1 month.

3.4.2 Inositol standard working solution (0.100 mg/mL): accurately transfer-take 5.00 mL of inositol standard stock solution, use 70% ethanol to reach a constant volume of 50 mL, and evenly mix it. Prepare it right before use.

4 Instruments and Equipment

4.1 Gas chromatograph: equipped with a hydrogen flame ionization detector.

4.2 Analytical balance: with a division value of 0.1 mg and 1 mg.

4.3 Centrifuge: with a speed ≥ 4,000 r/min.

4.4 Oven: with a temperature accuracy of ± 2 °C.

4.5 Constant-temperature water bath: with a temperature accuracy of ± 2 °C.

4.6 Rotary evaporator.

4.7 Vortex oscillator.

4.8 Ultrasonoscope.

4.9 Nitrogen blower.

5 Analytical Procedures

5.1 Specimen Preparation

5.1.1 Dissolution

Weigh-take 1 g of solid specimen or 12 g of liquid specimen (accurate to 1 mg) that has been evenly mixed into a 100 mL conical flask. For solid specimen, use 12 mL of 40 °C ~ 45 °C warm water to dissolve it, and perform ultrasonic extraction for 10 minutes. Transfer the above treated specimen solution into a 50 mL volumetric flask, use 95% ethanol to reach a constant volume to the scale, and evenly mix it; let it stand to precipitate for 20 minutes. If the specimen has lumpy, rather than flocculent precipitate, re-weigh the sample, and use 30 mL of 40 °C ~ 45 °C warm water to re-dissolve the sample, add acetonitrile to reach a constant volume to the scale and evenly mix it; let it stand to precipitate for 20 minutes. After the precipitation is completed, draw-take 10 mL of the supernatant, at a speed not lower than 4,000 r/min, centrifuge it for 5 min, then, accurately transfer-take 5.00 mL of the supernatant to a 25 mL rotary evaporation bottle or screw-top glass bottle and reserve it for drying.

5.1.2 Drying

Add an appropriate amount of absolute ethanol to the specimen to be dried, at a temperature not higher than 80 °C, use the rotary evaporator or nitrogen blower to concentrate it to near dryness. At 100 °C, bake it for 1 h. Take it out to cool to room temperature and reserve it for derivatization.

5.1.3 Derivatization

Add 10 mL of the silanization reagent to the dried specimen, conduct ultrasound for 5 minutes, seal and evenly mix it in a 25 mL screw-top glass bottle. In 80 °C water bath, react for 75 minutes, during which, take it out and oscillate once every 20 minutes. After it is over, cool to room temperature, add 5 mL of n-hexane and vortex for 2 minutes. Let it stand for stratification, then, take 3 mL of n-hexane extracting solution into a centrifuge tube that has been pre-added with a little anhydrous sodium sulfate, vortex, then, at a speed not lower than 4,000 r/min, centrifuge for 5 min. Then, transfer the solution into a sample injection bottle to obtain the specimen determination solution to be determined by the gas chromatograph.

5.2 Preparation of Inositol Standard Determination Solutions

Respectively draw-take 0.200 mL, 0.400 mL, 0.600 mL, 0.800 mL, 1.00 mL and 2.00 mL of inositol standard working solution (0.100 mg/mL) into rotary evaporation bottles or screw-top glass bottles. The other analytical procedures are the same as 5.1.2 and 5.1.3. The inositol content in the obtained standard determination solutions is respectively: 0.020 mg, 0.040 mg, 0.060 mg, 0.080 mg, 0.100 mg and 0.200 mg.

NOTE: the concentration range of the inositol standard determination solutions can be adjusted

Method II - Microbiological Method

9 Principle

Inositol is an essential nutrient for the growth of *Saccharomyces cerevisiae*. Under certain conditions, there is a corresponding relation between the growth of *Saccharomyces cerevisiae* and the inositol content. Taking the standard working curve as a reference, in accordance with the absorbance value of the solution to be tested, the inositol content in the specimen to be tested can be calculated.

10 Reagents and Materials

10.1 Equipment

10.1.1 Balance: with a division value of 0.1 mg, 1 mg and 0.1 g.

10.1.2 pH meter: with an accuracy of ± 0.01.

10.1.3 Spectrophotometer (at a wavelength of 550 nm).

10.1.4 Constant-temperature incubator: 30 °C ± 1 °C.

10.1.5 Oscillation incubator: 30 °C ± 1 °C, with an oscillation frequency of 140 r/min ~ 160 r/min.

10.1.6 High-pressure steam sterilizer: 121 °C (0.10 MPa ~ 0.12 MPa); 125 °C (0.13 MPa ~ 0.15 MPa).

10.1.7 Thermostat (or water bath): 100 °C ± 1 °C.

10.1.8 Centrifuge: with a speed ≥ 2,000 r/min.

10.1.9 Refrigerator: 2 °C ~ 5 °C.

10.1.10 Vortex oscillator.

10.1.11 Homogenizer.

10.2 Materials

10.2.1 Glass beads: with a diameter of about 5 mm.

10.2.2 Test tube: 18 mm × 180 mm.

10.2.3 Sterile pipette: 10 mL (with a scale of 0.1 mL) or 10 mL micropipette and tip.

10.2.4 Conical flask: 200 mL or 250 mL.

10.2.5 Volumetric flask (Type A): 50 mL, 100 mL and 250 mL.

10.2.6 Funnel: with a diameter of 90 mm.

10.2.7 Quantitative filter paper: with a diameter of 90 mm.

NOTE: before using the glass instrument, use an active agent (add sodium laurel sulfonate or household detergent to the washing water) to clean the hard glass measuring tube and other necessary glassware. After cleaning, dry heat at 200 °C for 2 hours.

11 Culture Media and Reagents

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-2 water specified in GB/T 6682.

11.1 Culture Media

11.1.1 Malt extract agar culture medium: see B.1 in Appendix B.

11.1.2 Malt extract liquid culture medium: see B.2 in Appendix B.

11.1.3 Culture medium for inositol determination: see B.3 in Appendix B.

NOTE: commercial synthetic media can be prepared in accordance with the instructions.

11.2 Reagents and Strain

11.2.1 Sodium chloride (NaCl).

11.2.2 Sodium hydroxide (NaOH).

11.2.3 Hydrochloric acid (HCl).

11.2.4 Phosphorus pentoxide (P_2O_5).

11.2.5 *Saccharomyces cerevisiae* ATCC 9080, or other validated equivalent standard strains.

11.3 Preparation of Reagents

11.3.1 Sterile sodium chloride solution (0.85%): weigh-take 8.5 g of sodium chloride and dissolve it in 1,000 mL of water, and divide it into test tubes, with 10 mL in each tube. At 121 °C, sterilize for 15 minutes.

11.3.2 Hydrochloric acid solution (1 mol/L): measure-take 90 mL of concentrated hydrochloric acid, reach a constant volume of 1,000 mL and evenly mix it.

11.3.3 Hydrochloric acid solution (0.44 mol/L): measure-take 39.6 mL of concentrated

hydrochloric acid, reach a constant volume of 1,000 mL and evenly mix it.

11.3.4 Sodium hydroxide solution (15 mol/L): weigh-take 300 g of sodium hydroxide and dissolve it in water. After cooling, reach a constant volume of 500 mL and evenly mix it.

11.3.5 Sodium hydroxide solution (1 mol/L): weigh-take 40 g of sodium hydroxide and dissolve it in water. After cooling, reach a constant volume of 1,000 mL and evenly mix it.

11.4 Reference Material

Inositol reference material ($C_6H_{12}O_6$, CAS: 87-89-8): purity ≥ 99%, or a standard substance certified by the state and awarded a reference material certificate.

11.5 Preparation of Standard Solutions

11.5.1 Inositol standard stock solution (0.2 mg/mL): place inositol reference material in a phosphorus pentoxide desiccator to dry for more than 24 hours, weigh-take 50 mg (accurate to 0.1 mg) of the above-mentioned inositol reference material into a 100 mL beaker, use water to dissolve it, then, transfer it to a 250 mL brown volumetric flask. Dilute to the scale and evenly mix it. Store it at 2 °C ~ 8 °C. It shall remain valid for 1 month.

11.5.2 Inositol standard intermediate solution (10 μg/mL): accurately transfer-take 5.00 mL of inositol standard stock solution, use water to reach a constant volume in a 100 mL brown volumetric flask. Store it at 2 °C ~ 8 °C. Prepare it right before use.

11.5.3 Inositol standard working solution (1 μg/mL and 2 μg/mL): accurately transfer-take 10.00 mL of inositol standard intermediate solution twice, respectively use water to reach a constant volume in a 100 mL brown volumetric flask and a 50 mL brown volumetric flask. Prepare it right before use.

12 Analytical Procedures

12.1 Preparation of Strain

12.1.1 Strain recovery

Inoculate *Saccharomyces cerevisiae* onto the slant of the malt extract agar culture medium, at 30 °C ± 1 °C, culture it for 16 h ~ 24 h. Then, transplant 2 ~ 3 generations to enhance the activity and prepare a stock strain. Store it in the refrigerator at 4 °C. The storage period shall not exceed 2 weeks.

12.1.2 Preparation of bacterial suspension

Before use, inoculate the stock strain onto the slant of a new malt extract agar culture medium, at 30 °C ± 1 °C, culture it for 16 h ~ 24 h. Then, transplant one ring of the slant culture into 10 mL of malt extract liquid culture medium, at 30 °C ± 1 °C, culture it for 20 h ~ 24 h. Thoroughly oscillate and mix the above-mentioned 10 mL fresh culture, transfer it to

a centrifuge tube, at 2,000 r/min, centrifuge it for 15 min, and discard the supernatant. Add 10 mL of sterile 0.85% sodium chloride solution, mix and re-suspend it. Repeat the centrifugation and re-suspension steps twice to prepare a 10 mL bacterial suspension and reserve it for later use.

Take sterile 0.85% sodium chloride solution as a blank, use a spectrophotometer to determine the light transmittance of the bacterial suspension at a wavelength of 550 nm. Adjust the concentration of the bacterial suspension, so that the light transmittance is 60% ~ 80%, and use it within 1 hour.

12.2 Preparation and Extraction of Specimens

Solid specimens, for example, cereals, need to be crushed, ground and sieved (the sieve plate has an aperture of 0.3 mm ~ 0.5 mm); for specimens such as meat and meat products, use a homogenizer to make them into chyme; specimens such as fruits and vegetables need to be homogenized and evenly mixed; for liquid specimens, shake and mix them before determination. Specimens are prepared right before use.

Accurately weigh-take an appropriate amount of specimen, preferably containing 0.5 mg ~ 2.0 mg of inositol. Generally, for foods with a relatively high inositol content, such as: fresh fruits and vegetables, offal and raw meat, weigh-take 1 g (accurate to 0.001 g); for foods with a relatively low inositol content, such as: cereals and beans, weigh-take 5 g (accurate to 0.001 g); for general nutrient supplements and compound nutritional fortifiers, weigh-take 0.1 g ~ 0.5 g (accurate to 0.001 g); for liquid beverages or liquid or semi-liquid specimens, weigh-take 5 g ~ 10 g (accurate to 0.001 g), and place it in a 250 mL conical flask. For solid specimens, add 80 mL of hydrochloric acid solution (0.44 mol/L); for liquid or semi-solid specimens, add 100 mL of hydrochloric acid solution (0.44 mol/L) and evenly mix them.

Use an aluminum foil to cover the conical flask and place it in a high-pressure steam sterilizer for high-pressure hydrolysis at 125 °C for 1 hour. Take it out, cool to room temperature, add about 2 mL of sodium hydroxide solution (15 mol/L) and cool it. Use sodium hydroxide solution (1 mol/L) or hydrochloric acid solution (1 mol/L) to adjust pH to 5.2 ± 0.1, transfer it to a volumetric flask of a certain volume V (adjusted in accordance with the inositol content in the sample, generally, 250 mL). Use water to reach a constant volume to the scale. Evenly mix it, use a filter paper to filter it and collect the filtrate. If necessary, further adjust the dilution factor f, so that the concentration of inositol in the solution to be tested is within the range of 0.1 μg/mL ~ 1.0 μg/mL; take it as the specimen extracting solution.

12.3 Preparation of Standard Solution

In accordance with Table 2, respectively add water, inositol standard working solution and inositol determination culture medium into the culture tubes. Prepare 3 portions of each tube in parallel.

at 100 °C, maintain for 5 min.

12.8.2 After using a vortex oscillator to thoroughly mix the culture in the test tubes, immediately transfer the culture solution into a cuvette for determination. After stabilizing for 30 seconds, read the absorbance. The stabilization time of each test tube shall be the same. Use S1 as a blank, adjust zero and read the absorbance of S2. Then, use S2 as a blank, adjust zero and successively read the absorbance of the remaining test tubes. If there is obvious bacterial growth in the uninoculated blank control tube S1, it indicates that there may be contamination by miscellaneous bacteria and the test needs to be re-performed. At each concentration point of the standard curve, the absorbance value measured of each tube shall not exceed ±15% of the average value. Take the inositol concentration as the x-coordinate and the absorbance as the y-coordinate, adopt the four-parameter Logistic curve fitting mode to fit the standard working curve. See the fitting equation in Appendix C.

NOTE: the four-parameter Logistic curve fitting adopts validated data analysis software.

12.8.3 In accordance with the absorbance of the solution to be tested, calculate the concentration of inositol in the solution to be tested from the standard working curve. Test values whose absorbance exceeds the range of standard curve tubes S3 ~ S10 shall be discarded. Calculate the concentration of inositol in three test tubes of each numbered (the test solution test tube No. 1 ~ 4 in Table 3, the same below) test solution, and compare it with the average value. The concentration measured of each test tube shall not exceed ±15% of the average value, and any excess shall be discarded (the total number of test tubes participating in the final statistics shall be greater than or equal to 8, otherwise, the result is invalid, and the test needs to be re-performed). Re-calculate the average value of inositol concentration in the test tubes of each numbered remaining test solution, respectively convert it to the concentration of inositol in the specimen extracting solution corresponding to each number, then, calculate the average concentration and record it as ρ. Use Formula (2) to calculate the inositol content X in the specimen.

NOTE: inositol test kits that have the same test principle as this Standard and has passed the equivalence verification can also be used.

13 Expression of Analysis Results

The content of inositol in the specimen is calculated in accordance with Formula (2).

$$X = \frac{\rho \times V \times f \times 100}{m \times 1\,000} \qquad \cdots\cdots (2)$$

Where,

X---the content of inositol in the specimen, expressed in (mg/100 g);

ρ---the total average mass concentration of inositol in the specimen extracting solution calculated in 12.8.3, expressed in (μg/mL);

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