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National food safety standards -- Determination of dietary fiber in food

食品安全国家标准 -- 食品中膳食纤维的测定

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National food safety standards -- Determination of dietary fiber in food

1 Scope

This Standard specifies the method for determination of dietary fiber in food.

This Standard is applicable to the determination of total dietary fiber, soluble dietary fiber, and insoluble dietary fiber in plant foods and their products, as well as foods with added dietary fiber components.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1 dietary fiber; DF

Carbohydrate polymers that cannot be digested and absorbed by the human small intestine and have a degree of polymerization ≥3.

According to its source, dietary fiber is divided into: Carbohydrate Polymers naturally present in the edible parts of plants, such as cellulose, hemicellulose, pectin, lignin, etc. in plant cell walls; & Carbohydrate Polymers that are isolated, extracted or synthesized from food raw materials using physical, enzymatic or chemical means and have been proven by scientific evidence to have beneficial physiological effects.

2.2 soluble dietary fiber; SDF

The portion of dietary fiber that is soluble in water, including indigestible oligosaccharides and some polysaccharides.

During the detection process, based on whether it can be precipitated by 78% ethanol, it is divided into precipitable soluble dietary fiber (SDFP) and non-precipitable soluble dietary fiber (SDFS).

2.3 insoluble dietary fiber; IDF

The portion of dietary fiber that is insoluble in water.

2.4 total dietary fiber; TDF

The sum of soluble dietary fiber and insoluble dietary fiber.

3 Principle

The specimen is homogenized and enzymatically hydrolyzed to remove starch and protein to obtain an indigestible enzymatic hydrolyzate.

The enzymatic hydrolyzate is precipitated with 78% ethanol. Collect the precipitated part, wash, dry and weigh it, and then measure the mass of DF (including IDF and SDFP) in the residue. Collect the filtrate portion. After desalting and concentration, liquid chromatography (internal standard method) is used to determine SDFS. The sum of the two is TDF.

The enzymatic hydrolyzate is filtered directly and washed with hot water. The filter residue is collected, washed, dried, and weighed to determine the mass of the IDF residue. The filtrate is collected and precipitated with 78% ethanol. The sediment is dried and weighed. The mass of the SDFP residue is determined. The filtrate fraction is measured as SDFS. The sum of SDFS and SDFP is SDF.

The mass of TDF, IDF and SDFP residues needs to be deducted from the mass of residual protein, ash and reagent blank to obtain the dietary fiber content of the corresponding part.

4 Reagents and materials

Unless otherwise stated, the reagents used in this method are analytically pure, and the water is grade two water specified in GB/T 6682.

4.1 Reagents

4.1.1 95% ethanol (CH_3CH_2OH).

4.1.2 Acetone (CH_3COCH_3).

4.1.3 Petroleum ether: boiling range 30℃~60℃.

4.1.4 Sodium hydroxide (NaOH).

4.1.5 Potassium dichromate ($K_2Cr_2O_7$).

4.1.6 Trishydroxymethylaminomethane ($C_4H_{11}NO_3$, Tris).

4.1.7 Maleic acid ($C_4H_4O_4$).

4.1.8 Concentrated hydrochloric acid (HCl).

4.1.9 Glacial acetic acid ($C_2H_4O_2$).

4.2.4 Hydrochloric acid solution (2 mol/L): Measure 167 mL of concentrated hydrochloric acid. Slowly add 500 mL of water. Mix well and add water to dilute to 1 L.

4.2.5 Sodium hydroxide solution (4 mol/L): Weigh 16 g of sodium hydroxide. Slowly add 60 mL of water. After dissolving, add water to dilute to 100 mL and mix well.

4.2.6 Sodium hydroxide solution (1 mol/L): Weigh 4 g of sodium hydroxide. Slowly add 60 mL of water. After dissolving, add water to dilute to 100 mL and mix well.

4.2.7 Sodium hydroxide solution (0.1 mol/L): Weigh 0.4 g of sodium hydroxide. Slowly add 60 mL of water. After dissolving, add water and dilute to 100 mL. Mix well.

4.2.8 Maleic acid buffer (50 mmol/L): Weigh 11.6 g of maleic acid and dissolve it in 1600 mL of water. Adjust to pH=6.0 with 4 mol/L sodium hydroxide solution. Add another 0.6 g of calcium chloride dihydrate. Add water to dilute to 2 L. Store at 4°C protected from light. The storage period does not exceed 1 month.

4.2.9 Trishydroxymethylaminomethane (Tris) solution (0. 75 mol/L): Weigh 90.8 g of Tris solid and dissolve it in about 800 mL of water. Add water to dilute to 1 L.

4.2.10 Acetic acid solution (2 mol/L): Measure 115 mL of glacial acetic acid. Add water to dilute to 1 L.

4.2.11 Mixed enzyme solution: Take 0.5 g of pancreatic α-amylase and 0.05 g of amyloglucosidase. Use 50 mL of 50 mmol/L maleic acid buffer to prepare a solution containing 400 U of pancreatic α-amylase and 30 U of amyloglucosidase per mL. Vortex for 5 min. Prepare when needed.

NOTE: If you need to reduce the amylase hydrolysis time, you can prepare a high-concentration mixed enzyme solution, that is, the concentrations of pancreatic α-amylase and amyloglucosidase are 800 U/mL and 340 U/mL respectively.

4.2.12 Protease solution: Take 2.5 g of protease. Use 50 mL of 50 mmol/L maleic acid buffer to prepare a protease solution containing 50 mg per mL. Vortex for 5 min. Prepare when needed. Store at 4°C before use.

4.2.13 Pickling diatomite: Take 200 g of diatomite in 600 mL of 2 mol/L hydrochloric acid. Soak overnight. Filter. Wash with water until the filtrate is neutral. Place it in a muffle furnace at 550℃±5℃ and burn it before use.

4.2.14 Potassium dichromate lotion: Weigh 100 g of potassium dichromate. Dissolve in 200 mL of water. Place the beaker in cold water to cool, then slowly add 1800 mL of concentrated sulfuric acid and mix. Stir with a glass rod while adding. Protect against spills.

4.3 Standard product

4.3.1 Diethylene glycol ($C_4H_{10}O_3$): CAS No. 111-46-6, purity ≥99%.

4.3.2 D-glucose ($C_6H_{12}O_6$): CAS No.50-99-7, purity ≥99%.

4.3.3 Fructose ($C_{18}H_{32}O_{16}$): CAS No. 470-69-9, purity ≥98%.

4.3.4 D-Maltose monohydrate ($C_{12}H_{22}O_{11} \cdot H_2O$): CAS No. 69-79-4, purity ≥98%.

4.4 Preparation of standard solution

4.4.1 Diethylene glycol internal standard solution (100 mg/mL): Accurately weigh 10 g (accurate to 0.1 mg) of diethylene glycol. Dilute with water. Transfer to a 100 mL volumetric flask. Add water and bring volume to the mark. Prepare when needed.

4.4.2 D-glucose standard solution (10 mg/mL): Accurately weigh 1.0 g (accurate to 0.1 mg) of D-glucose. Dissolve in water and transfer to a 100 mL volumetric flask. Add water to bring volume to the mark. Prepare when needed.

4.4.3 D-glucose/diethylene glycol solution standard series working solution: Pipette 0.50 mL, 1.0 mL, 2.0 mL, 4.0 mL and 8.0 mL of 10 mg/mL D-glucose standard solution into 10 mL volumetric flasks. Then add 0.2 mL of 100 mg/mL diethylene glycol internal standard solution. Add water to dilute and bring volume to the mark. The D-glucose contents in the standard series of working solutions are equivalent to 0.5 mg/mL, 1.0 mg/mL, 2.0 mg/mL, 4.0 mg/mL and 8.0 mg/mL respectively. Diethylene glycol content is equivalent to 2.0 mg/mL. Prepare when needed.

4.4.4 Qualitative standard solution: Weigh about 0.10 g of fructose and 0.10 g of D-maltose monohydrate. Dissolve in water. Transfer to a 50 mL volumetric flask. Add 1 mL of 100 mg/mL diethylene glycol internal standard solution. Add water to bring volume to the mark. Prepare when needed.

5 Instruments and equipment

5.1 Analytical balance: division is 0.1 mg and 1 mg.

5.2 Dietary fiber measuring device

5.2.1 Vacuum filtration device: Vacuum pump or aspirator with regulating device. 1 L suction filter bottle with a suction filter port on the side wall and a rubber stopper matching the suction filter bottle, used for suction filtration of enzymatic hydrolyzate.

5.2.2 Constant temperature oscillating water bath: with automatic timer. The temperature control range is room temperature +5℃~100℃. The temperature fluctuation is ±1℃.

5.2.3 Tall beaker without diversion port: 400 mL or 600 mL.

desiccator for later use.

NOTE: Users can also refer to GB 5009.3 to use the reduced pressure drying method.

6.1.3 Degreasing

For specimens with fat content ≥10%, weigh an appropriate amount of specimen (m_C, no less than 50 g) and place it in a 1000 mL Erlenmeyer flask. Add 500 mL of petroleum ether, mix and deflate. Shake for 2 min. After leaving it for 10 min, remove the petroleum ether three times in a row. After degreasing, the specimen is mixed and dried according to 6.1.2. Weigh (m_D). Calculate the specimen mass change factor (f) after treatment. After the specimen is homogenized, it is placed in a desiccator for later use.

NOTE: If the fat content of the specimen is unknown, it can be processed by first degreasing and then drying and grinding.

6.1.4 Desugarization

Weigh an appropriate amount of specimen (m_C, not less than 50 g). Place in a 1000 mL Erlenmeyer flask. Add 500 mL of 85% ethanol solution. Mix well. Shake for 2 min. Remove the ethanol solution portion, 3 times in a row. After desugarization, the specimen is dried in an oven at 40°C overnight. Weigh (m_D). Calculate the specimen mass change factor (f) after treatment. After the specimen is homogenized, it is placed in a desiccator for later use.

NOTE: Generally, the specimen does not need to be desugarized. If the viscosity of the specimen is too high due to high sugar content, which will affect the subsequent enzymatic hydrolysis and suction filtration effects, desugaring treatment shall be used. Specimens that need to be measured for SPFS shall not be desaccharified.

6.2 Specimen weighing

Accurately weigh 2 specimens (m) to be tested, accurate to 0.1 mg. The mass difference between the two specimens is ≤0.005 g. Generally, 0.25 g~3 g of solid specimen is weighed. Weigh 1.0 g~5.0 g of liquid specimen.

6.3 Enzymatic hydrolysis

Transfer the specimen to a 400 mL~600 mL tall beaker. Add 35 mL of 50 mmol/L maleic acid buffer. Stir magnetically until the specimen is completely dispersed in the buffer. Prepare 2 blank samples simultaneously and operate simultaneously.

NOTE: Stir evenly to avoid the specimen from forming clumps to prevent the specimen from fully contacting the enzyme during enzymatic hydrolysis.

6.3.1 Enzymatic hydrolysis of amylase

6.3.1.1 Enzymatic hydrolysis conditions 1 (applicable to specimens that do not contain

resistant starch)

Heat-stable α-amylase enzymatic hydrolysis: Add 50 μL of heat-stable α-amylase solution into a tall beaker. Stir slowly. Cover with aluminum foil. Place in a constant temperature oscillating water bath at 95℃~100℃. Start timing when the temperature rises to 95°C, and shake the reaction for 35 min. Take the beaker out. Cool to 60°C. Open the foil lid. Use a spatula to scrape off the paste from the inner wall of the beaker and the gel from the bottom of the beaker. Rinse the beaker wall and spatula with 5 mL of 50 mmol/L maleic acid buffer.

NOTE: To help starch disperse, 10 mL~15 mL of dimethyl sulfoxide can be added appropriately.

Amyloglucosidase enzymatic hydrolysis: Add 100 μL of amyloglucosidase solution to a tall beaker while stirring. Cover with aluminum foil. Continue to place in 60℃±1℃ water bath. When the water temperature reaches 60°C, time the reaction and shake for 30 min.

6.3.1.2 Enzymatic hydrolysis conditions 2 (applicable to all specimens)

Add 5 mL of pancreatic α-amylase and amyloglucosidase mixed enzyme solution to a tall beaker. Stir slowly. Cover with aluminum foil. Place in a constant temperature shaking water bath at 37°C and continue shaking. Start timing when the temperature rises to 37°C, and proceed to enzymatic hydrolysis for 16 h.

NOTE: If a high-concentration mixed enzyme solution is used, the enzymatic hydrolysis time can be appropriately shortened to no less than 4 h.

Open the foil lid. Add 3.0 mL of 0.75 mol/L Tris solution to the specimen solution to bring the pH of the specimen solution to 8.2±0.2. Cover with aluminum foil. Place in a water bath at 95℃~100℃ and heat for about 20 min. Shake the beaker gently from time to time. Take out the beaker and cool to 60℃±1℃.

6.3.2 Enzymatic hydrolysis of protease

Add 100 μL of protease solution to each beaker (for animal foods, add 500 μL of protease solution). Cover with aluminum foil. Place in a 60℃±1℃ water bath and continue shaking for 30 min. Open the foil lid. Add 4 mL of 2 mol/L acetic acid solution while stirring. Use 1 mol/L sodium hydroxide solution or 1 mol/L hydrochloric acid solution to adjust the pH to 4.3±0.2.

NOTE: Be sure to adjust the pH at 60℃±1℃, because lowering the temperature will increase the pH. Pay attention to the pH measurement of the blank sample. Make sure the pH is within the appropriate range.

6.3.3 Addition of internal standard solution

For example, when measuring SDFS, add 2 mL of 100 mg/mL diethylene glycol internal standard solution to the enzymatic solution. Mix well.

anion exchange resin (OH^-) and hydrogen ion exchange resin (H^+). Take 5 mL of reconstituted solution and add it to the polypropylene tube. Tighten the lid and mix repeatedly by inverting for more than 5 min to desalt. Let stand for 10 min. Transfer the supernatant to a 15 mL capped polypropylene tube. Add 5 mL of water to the pellet. Tighten the lid and mix by inverting repeatedly. Let stand for more than 5 min. Combine supernatants. After mixing, filter through a 0.45 μm filter membrane and put on the machine for testing.

6.4.4.4 Chromatographic reference conditions

The chromatographic reference conditions are as follows:

a) Chromatography column: High-efficiency aqueous size exclusion (SEC) gel chromatography column, using hydrophilic spherical porous polymethacrylate polymer as filler. Pore diameter is less than 20 nm. The column length is 300 mm. Inner diameter 7.8 mm. Particle size 7 μm, or equivalent column. Two gel chromatography columns in series;

b) Guard column: High performance aqueous size exclusion (SEC) gel guard column. The packing material is the same as the column. The column length is 40 mm. Inner diameter is 6.0 mm. Particle size is 12 μm;

c) Mobile phase: water (grade one water);

d) Column temperature: 80℃;

e) Detector temperature: 50℃;

f) Injection volume: 20 μL;

g) Flow rate: 0.5 mL/min;

h) Elution time: 60 min.

6.4.4.5 Determination of SDFS retention time: Take the qualitative standard solution and measure it on the machine. Measure at least 2 times in parallel. Based on the retention time and baseline separation effect of sucrose and D-maltose, determine the cutoff value for carbohydrate polymerization degree ≥ 3 and polymerization degree < 3, and the retention time interval of the component to be tested. Chromatograms can be found in Annex B.

6.4.4.6 Determination of D-glucose response factor (Rf): Take the D-glucose/diethylene glycol standard series working solution and measure it on the machine. Taking the ratio of D-glucose mass to diethylene glycol mass in the standard series of working solutions as the abscissa, and taking the ratio of the D-glucose peak area to the internal standard diethylene glycol peak area as the ordinate, draw a standard curve past the (0,0) point. The slope of the curve is the response factor (Rf).

6.4.4.7 SDFS determination: Take the desalted specimen liquid and measure it on the machine. The SDFS content is calculated based on the peak area of polymer with degree of polymerization ≥3 (PASDFS) and the peak area of diethylene glycol (PA_{IS}).

6.5 Determination of insoluble dietary fiber (IDF)

6.5.1 Weigh the sample according to 6.2. Carry out enzymatic hydrolysis according to 6.3.

6.5.2 Suction filtration: Take the treated crucible. Moisten the diatomaceous earth with 3 mL of water and flatten it. Remove the water and spread the diatomaceous earth in the crucible on the filter plate. Transfer all the specimen enzymatic hydrolyzate to the crucible and filter with suction. The residue is washed twice with 10 mL of 70°C hot water and used for the determination of IDF.

6.5.3 Residue determination: Operate according to 6.4.3.

6.6 Determination of soluble dietary fiber (SDF)

6.6.1 Weigh the sample according to 6.2. Carry out enzymatic hydrolysis according to 6.3.

6.6.2 Suction and filter according to 6.5.2.

6.6.3 Collect the filtrate: Collect the filtrate into another pre-weighed 600 mL tall beaker. Weigh the total mass of "beaker + filtrate". Deduct the mass of the beaker. Estimate the filtrate volume.

6.6.4 Precipitation: Add 95% ethanol preheated to 60℃±1℃ at a ratio of 4:1 between ethanol and filtrate volume. Cover with aluminum foil. Precipitate at room temperature for more than 1 h.

6.6.5 Suction-filtration one more time: Operate according to 6.4.2. The filtrate is used for the determination of SDFS, and the residue is used for the determination of SDFP.

6.6.6 Determination: Determine SDFP of the residue according to 6.4.3. Determine SDFS of the filtrate according to 6.4.4. The sum of SDFP and SDFS is soluble dietary fiber.

NOTE: For specimens without added dietary fiber components, the soluble dietary fiber does not need to contain the SDFS part.

6.7 Expression of analysis results

6.7.1 The mass change factor during specimen preparation is calculated according to formula (1).

Annex A

Definition of enzyme activity units and criteria for determining enzyme activity

A.1 Definition of enzyme activity units

A.1.1 Pancreatic α-amylase activity

Refer to the amylase activity tested with p-nitrophenyl maltose as substrate. 1 Enzyme activity unit (U) is: the amount of enzyme required to release 1.0 mg of maltose from starch every 3 mins at 20℃ and pH =6.9.

A.1.2 Thermostable α-amylase activity

Refer to the amylase activity tested with p-nitrophenyl maltose as substrate. 1 Enzyme activity unit (U) is: the amount of enzyme required to release 1.0 mg of maltose from starch every 3 min at 20℃ and pH =6.9.

A.1.3 Protease activity

Refer to the protease activity tested using casein as substrate. 1 Enzyme activity unit (U) is: the amount of enzyme required to hydrolyze a certain amount of tyrosine (equivalent to the color change caused by 1.0 μmol tyrosine in the color development reaction; Folin-Ciocalteau reagent is used for color development) from casein every 1 min at 37°C and pH 7.5.

A.1.4 Amyloglucosidase

Refer to the amyloglucosidase activity tested by the starch/glucose oxidase-peroxidase method. One unit of enzyme activity (U) is: the amount of enzyme required to release 1 mg of glucose from starch every 3 min at 55℃ and pH=4.5.

A.2 Criteria for determining enzyme activity

For enzyme reagents from different sources or different production batches, or enzyme reagents that have not been used for more than 6 months, the enzyme activity can be measured as listed in Table A.1 to help calibrate the enzyme dosage to achieve the expected effect of enzymatic hydrolysis, and eliminate interference from other enzymes.

Accurately weigh the standard substrate of corresponding mass. Add the corresponding enzyme solution according to the dosage in 6.3 for enzymatic hydrolysis. After enzymatic hydrolysis for a certain period of time, determine the substrate standard content. Recovery rate = (mass of substrate standard after enzymatic hydrolysis/mass of substrate standard before enzymatic hydrolysis) × 100%. It can be used normally if

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