GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.89-2016

National food safety standard

Determination of niacin and nicotinamide in foods

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China Food and Drug Administration.

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Foreword

The Standard replaces GB/T 5009.89-2003 “Determination of niacin in foods”, GB 5413.15-2010 “National food safety standard - Determination of vitamin niacin and niacinamide in foods for infants and young children, milk and milk products” and GB/T 9695.25-2008 “Meat and meat products - Determination of vitamin PP content”.

Compared with GB 5413.15-2010, the main changes of this Standard are as follows:

- The standard name is changed to “National food safety standard - Determination of niacin and nicotinamide in foods”;
- ADJUST the reagent sequence and format;
- MODIFY and REFINE the pre-treatment methods applicable to different food types (Method I);
- ADD the standard solution concentration calibration method (Method II);
- REASSESS the detection limit, ADD the limit of quantification.

National food safety standard –

Determination of niacin and nicotinamide in foods

1 Scope

This Standard specifies the determination method for niacin and nicotinamide in foods.

In this Standard, method I is microbiological method, which is applicable for the determination of the total content of niacin and nicotinamide in all types of foods including fortified foods using natural foods as the base. Method II is high performance liquid chromatography, which is applicable for the determination of acid and nicotinamide in fortified foods.

Method I Microbiological method

2 Principle

Niacin (nicotinamide) is a nutrient necessary for the growth of *Lactobacillus plantarum* (ATCC 8014). Under certain control conditions, use the specificity of *Lactobacillus plantarum* to niacin and nicotinamide that forms the optical density in the sample containing niacin and nicotinamide, to determine the content of niacin and nicotinamide.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are analytical reagents, and the water is Grade 2 water specified in GB/T 6682. The medium can be commercialized medium that meets the test requirements.

3.1 Strains

Lactobacillus plantarum (ATCC 8014), or other valid standard strains.

3.2 Reagents

3.2.1 Hydrochloric acid (HCl).

3.2.2 Sodium hydroxide (NaOH).

3.2.3 Sodium chloride (NaCl).

stock strains.

The stock strain is inoculated into Lactobacillus agar medium before the test, and cultured in the incubator at 36 °C ± 1 °C for 20 h ~ 24 h to activate the strain for the preparation of inoculation solution. Stock strains that are stored more than a few weeks cannot be used immediately for the preparation of inoculation solution, they shall be continuously inoculated 2 generations to 3 generations before the test to ensure the vitality of the strain.

5.2 Preparation of inoculation solution

One day before the test, MOVE part of the strain from the Lactobacillus agar medium to 10 mL of sterilized Lactobacillus broth medium, and CULTURE in the incubator at 36 °C ± 1 °C for 6 h ~ 18 h. Under aseptic conditions, CENTRIFUGE the culture broth for 15 min and DISCARD the supernatant. ADD 10 mL of sterilized physiological saline to re-disperse the cells; MIX thoroughly on a vortex mixer; CENTRIFUGE for 15 min; DISCARD the supernatant. REPEAT the centrifugation and cleaning steps three times. PIPETTE 1 mL of third-time cell dispersion solution to add to 10 mL of sterilized physiological saline, so that it is mixed evenly into a suspension, for further use. The light transmittance value of the suspension is read with a 721 spectrophotometer at a wavelength of 550 nm and using 0.9 % saline as a reference. The light transmittance value is adjusted with 0.9 % physiological saline or third-time cell dispersion solution to be within 60 % to 80 %. USE immediately.

5.3 Preparation of samples

Potatoes, beans, nuts (shelled) samples shall be crushed, ground and sieved (the aperture of sieve plate is 0.3 mm ~ 0.5 mm); milk powder and rice flour samples shall be mixed well; meat, eggs, fish, animal viscera shall be made into chyme with crushing machine; fruits, vegetables and semi-solid foods samples shall be mixed homogeneously; liquid samples shall be shaken to mix well before use. If the sample cannot be tested immediately, it shall be stored in the refrigerator at 4 °C.

5.4 Extraction of samples

Accurately weigh niacin samples. WEIGH 2 g ~ 5 g (accurate to 0.01 g) of general dairy, fresh fruit and vegetable sample; WEIGH 0.2 g ~ 1 g (accurate to 0.01 g) of cereals, beans, nuts, viscera, raw meat, dry sample, 5 g of liquid sample; accurately WEIGH 2 g (accurate to 0.01 g) of milk powder, rice flour sample; WEIGH 0.1 g ~ 0.5 g of general nutrient supplement and complex nutrition enhancer; WEIGH 0.2 g ~ 1 g of food; WEIGH 5 g ~ 10 g of liquid beverage or liquid, semi-liquid sample in a 100-mL Erlenmeyer flask, adding sulfuric acid solution with mass 10 times the dry mass of the tested substance. After hydrolysis at 121 °C for 30 min, cool to room temperature. USE 0.1 mol/L sodium hydroxide solution to adjust the pH to 6.0 ~ 6.5, then USE 0.1 mol/L hydrochloric acid to adjust the pH to 4.5 ± 0.1, DILUTE with water to 100 mL, FILTER

calculate the content of niacin and nicotinamide in the sample.

10 Reagents and materials

Unless otherwise stated, the reagents used in this method are analytical regents and the water is Grade 1 water specified in GB/T 6682.

10.1 Reagents

10.1.1 Hydrochloric acid (HCl): guarantee reagent.

10.1.2 Sodium hydroxide (NaOH): guarantee reagent.

10.1.3 Perchloric acid ($HClO_4$): with volume fraction of 60 %, guarantee reagent.

10.1.4 Methanol (CH_3OH): chromatographically pure.

10.1.5 Isopropyl alcohol (C_3H_8O): chromatographically pure.

10.1.6 Sodium heptanesulfonate ($C_7H_{15}NaO_3S$): chromatographically pure.

10.1.7 Amylase: with enzyme activity ≥ 1.5 μ/mg.

10.2 Preparation of reagents

10.2.1 Hydrochloric acid (5.0 mol/L): MEASURE 415 mL of hydrochloric acid with a measuring cylinder in a 1000 mL flask; ADD 585 mL of water; MIX well.

10.2.2 Hydrochloric acid (0.1 mol/L): PIPETTE 8.3 mL of hydrochloric acid with a pipette in a 1000 mL flask; ADD 991.7 mL of water; MIX well.

10.2.3 Sodium hydroxide (5.0 mol/L): WEIGH 200 g of sodium hydroxide (3.2.2) in the flask and ADD water to dissolve; TRANSFER to a 1000-mL volumetric flask; ADD water to the constant volume; MIX well.

10.2.4 Sodium hydroxide (0.1 mol/L): WEIGH 4.0 g of sodium hydroxide (3.2.2) in the flask and ADD water to dissolve; TRANSFER to a 1000-mL volumetric flask; ADD water to the constant volume; MIX well.

10.2.5 Mobile phase: 70 mL of methanol, 20 mL of isopropanol and 1 g of sodium heptanesulfonate, that are dissolved with 910 mL of water and mixed well, with the pH adjusted to 2.1 ± 0.1 with perchloric acid, and filtered through 0.45 μm membrane.

10.3 Niacin and nicotinamide standard solution

Niacin ($C_6H_5NO_2$) and nicotinamide ($C_6H_6N_2O$): with purity > 99 %, or reference materials certified by the state and awarded the reference material certificate.

ADD about 25 mL of water at 45 °C to 50 °C; WEIGH about 20.0 g (accurate to 0.01 g) of the mixed homogeneous liquid sample in a 150-mL erlenmeyer flask; ADD about 0.5g amylase; SHAKE well; FILL the flask with nitrogen; PLUG the stopper; PLACE the flask in the incubator at 50 °C ~ 60 °C for about 30 min; REMOVE and COOL to room temperature.

NOTE: If conditions permit, it is recommended to oscillate in the water bath at 55 °C ± 5 °C during the hydrolysis.

12.1.2 Starch-free food (prepared milk, prepared milk powder, beverages, solid beverages, soy flour, soy milk powder, etc.)

WEIGH about 5.0 g (accurate to 0.01 g) of the mixed homogeneous solid sample, and ADD about 25 mL of water at 45 °C to 50 °C; WEIGH about 20.0 g (accurate to 0.01 g) of the mixed homogeneous liquid sample in a 150-mL erlenmeyer flask; PLACE the flask in the ultrasonic oscillator to oscillate for about more than 10 min to fully dissolve; STAND still for 5 min ~ 10 min; cool to room temperature.

12.1.3 Extraction

After the sample solution is dropped to room temperature, adjust the pH of the sample solution to 1.7 ± 0.1 with 5.0 mol/L hydrochloric acid solution and 0.1 mol/L hydrochloric acid solution. After standing still for about 2 min, adjust the pH of the sample solution to 4.5 ± 0.1 with 5.0 mol/L sodium hydroxide solution and 0.1 mol/L sodium hydroxide solution. PLACE the sample solution in 50 °C water bath ultrasonic oscillator to oscillate for more than 10 min to fully extract; COOL to room temperature; TRANSFER to a 100-mL volumetric flask; RINSE the flask with water repeatedly, and COLLECT the rinsing fluid in a 100-mL volumetric flask; DILUTE with water to the scale; MIX well; FILTER by filter paper. The filtrate is further filtered through a 0.45 μm microporous membrane and collected with a sample flask, that is the sample determination solution.

NOTE: If necessary, the sample determination solution is diluted with water (*f*), so that the concentration of niacin and nicotinamide in the sample determination solution is in the range of 1 μg/mL ~ 20 μg/mL.

12.2 Reference liquid chromatographic conditions

The reference liquid chromatographic conditions are listed below:

a) Chromatographic column: C_{18} (with particle diameter of 5 μm, 250 mm × 4.6 mm) or chromatographic column with equivalent performance;

b) Column temperature: 25 °C ± 0.5 °C;

c) UV detector: with detection wavelength of 261 nm;

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