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National Food Safety Standard - Determination of Niacin and Niacinamide in Foods

食品安全国家标准　食品中烟酸和烟酰胺的测定

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Table of Contents

National Food Safety Standard - Determination of Niacin and Niacinamide in Foods

1 Scope

This Standard specifies the methods for the determination of niacin and niacinamide in foods.

In this Standard, Method 1 is applicable to the determination of niacin and niacinamide in prepared milk powder, special dietary foods (excluding partially hydrolyzed milk protein formulas, deeply hydrolyzed milk protein formulas or amino acid formulas, and infant formulas for special medical purposes for amino acid metabolism disorders) and special-purpose beverages. Method 2 is applicable to the determination of niacin (or niacinamide) in foods.

Method I - High Performance Liquid Chromatography

2 Principle

After pre-treatment, such as: enzymatic hydrolysis and protein precipitation, the sample is extracted through ultrasonic oscillation in a weakly acidic environment, separated by C_{18} chromatographic column and detected by a UV detector. In accordance with the retention time of the chromatographic peak, conduct qualitative determination, and adopt the external standard method for quantitative determination. Calculate the content of niacin and niacinamide in the specimen.

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 Hydrochloric acid (HCl): guaranteed reagent.

3.1.2 Sodium hydroxide (NaOH): guaranteed reagent.

3.1.3 Methanol (CH_3OH): chromatographically pure.

3.1.4 Isopropyl alcohol (C_3H_8O): chromatographically pure.

3.1.5 Sodium heptane sulfonate ($C_7H_{15}NaO_3S$): chromatographically pure.

3.1.6 Amylase: enzyme activity ≥ 1.5 U/mg.

3.2 Preparation of Reagents

3.2.1 Hydrochloric acid solution (5.0 mol/L): measure-take 415 mL of hydrochloric acid and add water to reach a constant volume of 1,000 mL.

3.2.2 Hydrochloric acid solution (0.1 mol/L): draw-take 8.3 mL of hydrochloric acid and add water to reach a constant volume of 1,000 mL.

3.2.3 Sodium hydroxide solution (5.0 mol/L): weigh-take 200 g of sodium hydroxide and add water to reach a constant volume of 1,000 mL.

3.2.4 Sodium hydroxide solution (0.1 mol/L): draw-take 20 mL of sodium hydroxide solution (5.0 mol/L) and add water to reach a constant volume of 1,000 mL.

3.3 Reference Materials

3.3.1 Niacin ($C_6H_5NO_2$, CAS: 59-67-6): purity ≥ 98%, or a standard substance certified by the state and awarded a reference material certificate.

3.3.2 Niacinamide ($C_6H_6N_2O$, CAS: 98-92-0): purity ≥ 98%, or a standard substance certified by the state and awarded a reference material certificate.

3.4 Preparation of Standard Solutions

3.4.1 Niacin and niacinamide standard stock solution (1.000 mg/mL): place niacin (or niacinamide) reference material into a desiccator containing phosphorus pentoxide and dry it overnight. Respectively and accurately weigh-take 0.1 g (accurate to 1 mg), use 0.1 mol/L hydrochloric acid to dissolve it, and reach a constant volume of 100 mL. It can be stored for 1 month when refrigerated at 2 °C ~ 8 °C.

3.4.2 Niacin and niacinamide standard mixed intermediate solution (100.0 μg/mL): respectively and accurately draw-take 10.0 mL of niacin and niacinamide standard stock solution into a 100 mL volumetric flask, add water to reach a constant volume to the scale, and evenly mix it. It can be stored for 1 month when refrigerated at 2 °C ~ 8 °C.

3.4.3 Niacin and niacinamide standard mixed working solutions: respectively and accurately draw-take 1.0 mL, 2.0 mL, 5.0 mL, 10.0 mL and 20.0 mL of the standard mixed intermediate solution into 100 mL volumetric flasks, add water to reach a constant volume to the scale and evenly mix them. Thus, standard mixed working solutions with a mass concentration of 1.0 μg/mL, 2.0 μg/mL, 5.0 μg/mL, 10.0 μg/mL and 20.0 μg/mL are obtained. Prepare them right before use.

4 Instruments and Equipment

4.1 High performance liquid chromatograph: equipped with UV detector or diode array detector.

4.2 Balance: with a division value of 0.1 mg and 0.01 g, respectively.

4.3 Constant-temperature incubator: 30 °C ~ 80 °C.

4.4 Ultrasonic equipment.

4.5 pH meter: with an accuracy of 0.1.

4.6 Pulverizer.

5 Analytical Procedures

5.1 Sample Pre-treatment

5.1.1 Specimen preparation

Sample pre-treatment: take at least 200 g of representative sample. For lumpy or granular samples, use a pulverizer to pulverize them; for powdery, pasty or liquid samples, thoroughly mix them and place them in a closed container.

5.1.2 Starches and starch-containing foods

5.1.2.1 Weigh-take about 5.0 g (accurate to 0.01 g) of evenly mixed solid specimen, place it in a 150 mL conical flask, add about 25 mL of 45 °C ~ 50 °C water, add about 0.5 g of amylase, shake it well, then, fill the conical flask with nitrogen, use a stopper to cap it, and place it in an incubator at 50 °C ~ 60 °C for enzymatic hydrolysis for about 30 minutes. Take it out and cool to room temperature.

5.1.2.2 Weigh-take about 20.0 g (accurate to 0.01 g) of evenly mixed liquid specimen, place it in a 150 mL conical flask, add about 0.5 g of amylase, shake it well, then, fill the conical flask with nitrogen, use a stopper to cap it, and place it in an incubator at 50 °C ~ 60 °C for enzymatic hydrolysis for about 30 minutes. Take it out and cool to room temperature.

5.1.3 Starch-free foods

5.1.3.1 Weigh-take about 5.0 g (accurate to 0.01 g) of evenly mixed solid specimen, place it in a 150 mL conical flask, and add about 25 mL of 45 °C ~ 50 °C water. Place it in ultrasonic equipment and oscillate for more than 10 minutes to thoroughly dissolve it, let it stand for 5 minutes ~ 10 minutes, then, cool it to room temperature.

5.1.3.2 Weigh-take about 20.0 g (accurate to 0.01 g) of evenly mixed liquid specimen and place it in a 150 mL conical flask. Place it in ultrasonic equipment and oscillate for more than 10 minutes to thoroughly dissolve it, let it stand for 5 minutes ~ 10 minutes, then, cool it to room temperature.

5.1.4 Specimen extraction

After the specimen solution drops to room temperature, use 5.0 mol/L hydrochloric acid solution and 0.1 mol/L hydrochloric acid solution to adjust the pH of the specimen solution to

factor 1.008.

7 Precision

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

8 Others

For solid samples: when the sampling size is 5 g, the detection limit of niacin is 30 μg/100 g, and the quantitation limit is 100 μg/100 g; the detection limit of niacinamide is 40 μg/100 g, and the quantitation limit is 120 μg/100 g.

For liquid samples: when the sampling size is 20 g, the detection limit of niacin is 7.5 μg/100 g, and the quantitation limit is 25 μg/100 g; the detection limit of niacinamide is 10 μg/100 g, and the quantitation limit is 30 μg/100 g.

Method II - Microbiological Method

9 Principle

Niacin and niacinamide are essential nutrients for the growth of *Lactiplantibacillus plantarum*. In the niacin determination culture medium, the growth of *Lactiplantibacillus plantarum* is correlated with the niacin (or niacinamide) content. In accordance with the standard curve of niacin (or niacinamide) content and light transmittance (or absorbance), calculate the niacin (or niacinamide) content in the specimen.

10 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-1 or Grade-2 water specified in GB/T 6682.

10.1 Strain

Lactiplantibacillus plantarum (the former *Lactobacillus plantarum*) ATCC 8014, or equivalent strain.

10.2 Culture Media

10.2.1 Lactobacillus agar culture medium: see B.1 in Appendix B.

10.2.2 Lactobacillus broth culture medium: see B.2 in Appendix B.

10.2.3 Medium for niacin determination: see B.3 in Appendix B.

NOTE: commercially available synthetic media can be used and operated in accordance with the instructions.

10.3 Reagents

10.3.1 Absolute ethanol (C_2H_5OH).

10.3.2 Sulfuric acid (H_2SO_4): 95% ~ 98%.

10.3.3 Sodium hydroxide (NaOH).

10.3.4 Sodium chloride (NaCl).

10.4 Preparation of Reagents

10.4.1 Ethanol solution (with a volume fraction of 25%): measure-take 250 mL of absolute ethanol, add water to reach a constant volume of 1,000 mL.

10.4.2 Sulfuric acid solution A (10 mol/L): measure-take 560 mL of sulfuric acid, add it to water and dilute it to 1,000 mL.

10.4.3 Sulfuric acid solution B (0.5 mol/L): measure-take 50 mL of sulfuric acid A (10 mol/L), add it to water and dilute it to 1,000 mL.

10.4.4 Sodium hydroxide solution A (10 mol/L): weigh-take 400 g of sodium hydroxide, add water to dissolve it and dilute to 1,000 mL.

10.4.5 Sodium hydroxide solution B (0.1 mol/L): draw-take 10 mL of sodium hydroxide solution A (10 mol/L), add water to dilute it to 1,000 mL.

10.4.6 Sterile physiological saline (8.5 g/L): weigh-take 8.5 g of sodium chloride and dissolve it in 1,000 mL of distilled water, divide it into stoppered test tubes, with 10 mL in each tube. At 121 °C, perform autoclaved sterilization for 15 min.

10.5 Reference Materials

10.5.1 Niacin ($C_6H_5NO_2$, CAS: 59-67-6): purity ≥ 98%, or a standard substance certified by the state and awarded a reference material certificate.

10.5.2 Niacinamide ($C_6H_6N_2O$, CAS: 98-92-0): purity ≥ 98%, or a standard substance certified by the state and awarded a reference material certificate.

10.6 Preparation of Standard Solutions

10.6.1 Niacin (or niacinamide) standard stock solution (50 μg/mL): place niacin (or niacinamide) reference material into a desiccator containing phosphorus pentoxide and dry it overnight. In accordance with purity, weigh it, so that the niacin (or niacinamide) content is

11.14 Graduated pipette: 5 mL (with a scale of 0.1 mL).

11.15 Glass funnel: with a diameter of 100 mm.

11.16 Conical flask: with a capacity of 250 mL.

11.17 Beaker: with a capacity of 100 mL.

11.18 Dispenser: 0 mL ~ 10 mL.

11.19 Micropipette: 1,000 μL and 200 μL.

11.20 Sterile centrifuge tube: 1.5 mL.

11.21 Syringe filter: with an aperture of 0.22 μm.

NOTE: the cleaned glassware and metal appliances shall be dry-heated at 250 °C for 1 h ~ 2 h.

12 Analytical Procedures

12.1 Preparation of Test Bacterial Suspension

12.1.1 After the *Lactiplantibacillus plantarum* strain is activated, use an inoculating needle to pierce and inoculate it onto the lactobacillus agar culture medium, at 36 °C ± 1 °C, culture for 16 h ~ 24 h. Transplant 2 ~ 3 generations to enhance the vitality. Store it in the refrigerator as a slant culture. It shall remain valid for 1 month.

12.1.2 Transplant the strain activated within 24 hours to sterilized lactobacillus broth, at 36 °C ± 1 °C, culture for 16 h ~ 24 h. At 3,000 r/min ~ 5,000 r/min, centrifuge it for 5 min, discard the supernatant, add 10 mL of physiological saline, and use a vortex mixer to oscillate the suspension, then, centrifuge it for about 5 minutes and discard the supernatant. After washing 2 ~ 3 times as before, add 10 mL of physiological saline and evenly oscillate it. Draw-taken an appropriate amount of the bacterial suspension into 10 mL of physiological saline and evenly mix it to prepare a test bacterial suspension.

12.1.3 Use physiological saline as a blank, use a spectrophotometer to determine the transmittance (% *T*) of the test bacterial suspension at a wavelength of 550 nm, and adjust the amount of the above-mentioned bacterial solution added, so that the transmittance of the test bacterial suspension is between 60% *T* and 80% *T*.

12.2 Specimen Extraction

Lumpy and granular specimens need to be crushed; powdered specimens, such as: milk powder and rice flour, need to be evenly mixed; fruits and vegetables, meat, eggs, fish and animal offal, etc., need to be made into chyme; semi-solid foods need to be homogenized and evenly mixed; liquid specimens shall be shaken and mixed before use.

12.2.1 Solid specimens: accurately weigh-take the specimen (accurate to 0.001 g) and place it in a conical flask. Specifically speaking, 2 g ~ 5 g of fresh fruit and vegetable specimens; 0.2 g ~ 1 g of cereals, beans, nuts, offal, raw meat and dried specimens; 2 g ~ 3 g of milk powder and rice flour specimens; 0.1 g ~ 0.5 g of general nutrient supplements and compound nutritional supplements; 0.2 g ~ 1 g of other foods.

12.2.2 Liquid beverages or liquid and semi-liquid specimens: weigh-take 5 g ~ 10 g (accurate to 0.001 g) of specimen (or use a one-mark pipette to draw-take an appropriate volume) and place it in a conical flask. Special-purpose beverages do not need to be processed in accordance with 12.2.3 after sample weighing. After weighing the sample, directly reach a constant volume of 100 mL (V); in accordance with 12.2.4, dilute it.

If the niacin (or niacinamide) content in the specimen is excessively low, the sampling size can be appropriately increased.

12.2.3 Add sulfuric acid solution B (in milliliters) that is 10 times the specimen mass (in grams), put the above-mentioned mixture into a pressure steam sterilizer, at 121 °C, hydrolyze it for 30 minutes, then, take it out and quickly cool to room temperature in a water bath. Use sodium hydroxide solution A and sodium hydroxide solution B to adjust pH to 4.5 ± 0.2, transfer it into a 250 mL (V_1) volumetric flask, and use water to reach a constant volume to the scale.

Use a quantitative filter paper to filter it. The first 10 mL of filtrate shall be discarded. Draw-take 5 mL (V_2) of the filtrate into a 100 mL beaker, add about 20 mL of water, and use sodium hydroxide solution B to adjust pH to 6.8 ± 0.2. Transfer it into a 100 mL (V) volumetric flask and add water to reach a constant volume to the scale.

12.2.4 Dilution: in accordance with the niacin (or niacinamide) content in the specimen, use water to appropriately dilute the extracting solution, so that the mass concentration of niacin (or niacinamide) in the specimen extracting solution after dilution is 5 ng/mL ~ 12 ng/mL.

12.3 Specimen Determination

12.3.1 Test tube culture method

12.3.1.1 Standard curve series of tubes

In accordance with the sequence listed in Table 1, add water, standard curve working solution and culture medium for niacin determination to the culture tube. Prepare 3 tubes for each number in Table 1. In uninoculated blank test tube (UN), inoculated blank test tube (IN) and standard series of tubes S1 ~ S8, the mass concentration of niacin (or niacinamide) is respectively: 0 ng/mL, 5 ng/mL, 10 ng/mL, 15 ng/mL, 20 ng/mL, 25 ng/mL, 30 ng/mL, 40 ng/mL and 50 ng/mL.

12.3.1.6 Determination

After the culture is completed, visually inspect each test tube. The culture solution in the uninoculated blank test tube (UN) shall be clear. There shall be a gradient difference in the absorbance of the culture solution in the standard curve series of tubes and the specimen series of tubes. If the uninoculated blank test tube (UN) is turbid, then, the determination shall be deemed as invalid.

12.3.1.6.1 Use the uninoculated blank test tube (UN) as a blank, adjust the spectrophotometer transmittance to 100% *T*, and read the reading of the inoculated blank test tube (IN). Then, use the inoculated blank test tube (IN) as the blank, adjust the transmittance to 100% *T*, and successively read the transmittance (% *T*) (or absorbance *A*) of other test tubes.

12.3.1.6.2 Use a vortex mixer to thoroughly mix each test tube (a drop of antifoaming agent can also be added), then, immediately transfer the culture solution into a cuvette, at a wavelength of 550 nm, perform colorimetric determination. After the reading is stable, read the light transmittance. The stabilization time of each test tube shall be the same. Successively read the transmittance of other test tubes. Culture tubes whose transmittance exceeds the concentration range of standard curve series of tubes S1 ~ S8 shall be discarded. Take the mass concentration of the niacin or niacinamide reference material as the x-coordinate and the transmittance as the y-coordinate to draw a standard curve.

NOTE: to draw a standard curve, absorbance *A* can also be used as the y-coordinate.

12.3.1.6.3 For the test tubes of each numbered test solution, use the transmittance or absorbance of each test tube to calculate the concentration of niacin (or niacinamide) in each milliliter of the specimen extracting solution, and calculate the average concentration of niacin (or niacinamide) in the numbered extracting solution. The concentration measured of each test tube shall not exceed ±15% of the average value, and any excess shall be discarded. If the number of tubes that comply with this requirement is less than 2/3 of the total number of tubes of all 4 numbered extracting solutions, the data used to calculate the specimen content is insufficient and the test needs to be re-performed. If the number of tubes that comply with this requirement is less than 2/3 of the original number of tubes, re-calculate the average value of niacin (or niacinamide) content per milliliter of extracting solution in each numbered valid specimen tube, and use this average value to calculate the total average value of all numbered specimen tubes as ρ. In accordance with Formula (3) and the dilution factor and sampling size, calculate the content of niacin (or niacinamide) in the specimen.

12.3.2 Microplate culture method

12.3.2.1 Standard curve series of centrifuge tubes

Filter and sterilize the niacin standard curve working solution into a sterile centrifuge tube under sterile conditions. In accordance with Table 3, prepare 3 sets of standard curve series of centrifuge tubes. The niacin (or niacinamide) mass concentration in uninoculated blank pore (UN), inoculated blank pore (IN) and S1 ~ S8 is the same as that in the test tube method.

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