NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.5-2025

Replacing GB 5009.5-2016

National food safety standard - Determination of protein in foods

食品安全国家标准 食品中蛋白质的测定

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Standardization Administration of the PRC

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Foreword

This Standard replaced GB 5009.5-2016 National Food Safety Standard - Determination of Protein in Foods. Compared with GB 5009.5-2016, the major changes of this Standard are as follows:

--- Modify the scope of the standard;

--- Modify Method I the sampling volume and standard titration solution concentration of the Kjeldahl method;

--- Add Method II the storage conditions and time of the standard solution and color developer;

--- Modify Method III the scope and detection limit of the combustion method;

--- Add Appendix B combustion method calibration curve;

--- Modify the expression of analysis results and Appendix C protein conversion coefficient table;

--- Modify the precision. National Food Safety Standard - Determination of Protein in Foods

1 Scope

GB 5009.5 is the Chinese national food safety method for determining protein in food, and it carries a history: the melamine scandal happened precisely because the standard method measured nitrogen and inferred protein from it, and a nitrogen-rich adulterant passed. The method is still nitrogen-based, because nothing else is practical at scale, but everything around it has been tightened. The standard gives the Kjeldahl method, both the classical and the automatic analyser versions, and the combustion (Dumas) method, and for each the principle, the reagents, the apparatus, the sample preparation, the digestion or combustion procedure, the titration or detection, the calculation with the nitrogen-to-protein conversion factor for each food category, the precision and the limit of quantification. The 2025 edition replaces GB 5009.5-2016 and takes effect in September 2025. Every protein figure on a Chinese nutrition label, and every protein specification in a supply contract, rests on this method.

This Standard specifies the method for determination of protein in foods. This Standard is applicable to the determination of protein in foods. Method I Kjeldahl Method

2 Principle

The protein in food is decomposed under catalytic heating conditions; and the produced ammonia combines with sulfuric acid to form ammonium sulfate. Alkalinization distillation frees the ammonia, which is absorbed by boric acid and titrated with sulfuric acid or hydrochloric acid standard titration solution. The nitrogen content is calculated based on the consumption of sulfuric acid or hydrochloric acid, and then multiplied by the conversion coefficient to obtain the protein content.

3 Reagents and Materials

Unless otherwise specified, all reagents used in this method are analytically pure, and water is Grade-3 water specified in GB/T 6682.

3.2 Preparation of reagents

3.2.1 Boric acid solution (20 g/L). Weigh 20 g of boric acid; dissolve in water and dilute to 1,000 mL.

3.2.2 Sodium hydroxide solution (400 g/L). Weigh 40 g of sodium hydroxide; dissolve in water; cool and dilute to 100 mL.

4 Instruments and Equipment

4.1 Analytical balance. With sensitivity of 1 mg.

4.2 Nitrogen determination distillation apparatus. as shown in Appendix A.

4.3 Pipette. 10 mL, 25 mL and 50 mL.

4.4 Nitrogen determination bottle. 100 mL, 250 mL and 500 mL.

4.5 Digestion furnace. >=420°C.

4.6 Semi-automatic Kjeldahl nitrogen analyzer or fully automatic Kjeldahl nitrogen analyzer.

4.7 Homogenizer.

5.2 Fully automatic or semi-automatic Kjeldahl method Weigh

0.2 g ~ 2 g of solid specimen, 2 g ~ 5 g of semi-solid specimen, (accurate to

0.001 g), 10 g (mL) ~ 25 g (mL) (equivalent to 30 mg ~ 40 mg nitrogen) of liquid specimen; and then add

0.4 g of copper sulfate, 6 g of potassium sulfate and 20 mL of sulfuric acid to the digestion furnace for digestion. When the temperature of the digestion furnace reaches 420 °C, continue digestion for at least 1 h.

6 Expression of Analysis Results

The protein content in the specimen is calculated according to Formula (1). When the protein content is >=1 g/100 g or 1 g/100 mL, the result shall be retained 3 significant figures; when the protein content is < 1 g/100 g or 1 g/100 mL, the result shall be retained 2 significant figures.

7 Precision

When the protein content in the sample is <=10 g/100 g or 10 g/100 mL, the absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 10% of the arithmetic mean.

8 Others

0.05 mol/L hydrochloric acid titration solution, the sample weight is

5.0 mL; and the detection limit of nitrogen in this method is

0.008 g/100 g or

0.008 g/100 mL. Method II Spectrophotometry

9 Principle

The protein in food is decomposed under catalytic heating conditions. The ammonia produced by the decomposition combines with sulfuric acid to form ammonium sulfate, which reacts with acetylacetone and formaldehyde in a sodium acetate-acetic acid buffer solution at pH

4.8 to form a yellow 3,5-diacetyl-2,6-dimethyl-1,4-dihydropyridine compound.

10 Reagents and Materials Unless otherwise specified, the reagents used in this method are analytically pure, and the water is Grade-3 water specified in GB/T 6682.

10.4.1 Standard ammonium sulfate stock solution (by nitrogen) (

1.0 g/L). Weigh 0.4720 g of ammonium sulfate dried at 105°C for 2 h; dissolve in water and dilute to 100 mL; and mix well. Each milliliter of this solution is equivalent to

1.0 mg of nitrogen. It can be stored at 4°C for 1 month.

10.4.2 Standard ammonium sulfate working solution (

0.10 mg/mL). Accurately pipette

10.00 mL of standard ammonium sulfate stock solution (

1.0 g/L) into a 100 mL volumetric flask; dilute to the mark with water; and mix well. Each milliliter of this solution is equivalent to

0.1 mg of nitrogen. Prepare it before use.

11 Instruments and Equipment

11.1 Spectrophotometer.

11.2 Electro-thermostatic water bath. 100°C ±1°C.

11.3 pH meter. With accuracy of 0.01. 11.4 10 mL stoppered glass colorimetric tube.