

Determination of Protein Content in Hygroscopic Foods Using a Water-Cooling-Free Fully Automatic Kjeldahl Nitrogen Analyzer

References and Executive Standards

- GB 5009.5-2025
- GB/T 33862-2017
- ISO 8968-1:2014 / IDF 20-1:2014
- AOAC Official Method 991.20

Testing Instrument

- N810 Water-Free Fully Automatic Kjeldahl Nitrogen Analyzer

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Introduction

In the quality inspection of the food industry, the physical properties of food samples (such as whether they contain high fat, high sugar, or are prone to moisture absorption and caking, etc.) have a crucial impact on the pretreatment method and final detection results. Among them, moisture-absorbing and caking-prone foods (such as milk powder, solid instant beverages, etc.) easily absorb moisture in the air, bringing great challenges to accurate sampling. The disorderly introduction of moisture will not only change the actual mass of the sample but also directly lead to weighing errors, thereby seriously affecting the accuracy and repeatability of the protein content determination results. To avoid the negative impact of moisture on experimental results, specific pretreatment control measures such as drying before weighing, rapid weighing, or determining the moisture content separately without drying the sample are usually adopted for such samples.

This experiment takes typical moisture-absorbing food — milk powder (a food made from fresh cow's milk or goat's milk, dried after removing almost all the water in the milk) as an example, using the classic Kjeldahl method to analyze its total nitrogen/crude protein content. This method is highly universal (applicable to a variety of organic nitrogen-containing samples), classic and reliable, and has a controllable blank value. Combined with the efficient processing of the water-free fully automatic Kjeldahl nitrogen analyzer, this report aims to provide a professional, reliable, and highly operable experimental scheme reference for the accurate determination of protein in moisture-absorbing foods.

Experimental Instruments and Reagent System

Core Instruments

- Nitrogen Analyzer: N810 Water-Free Fully Automatic Kjeldahl Nitrogen Analyzer or N710 Fully Automatic Kjeldahl Nitrogen Analyzer.
- Digestion Equipment: NDS-20 Graphite Digestion Furnace (20-position).

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- Auxiliary Equipment: Analytical balance (accurate to 0.1mg).

Experimental Reagents

All reagents must meet analytical grade requirements:

- Sulfuric acid (analytical grade).
- 20g/L boric acid solution, used as the absorption liquid.
- Bromocresol green-methyl red mixed indicator.
- 400g/L sodium hydroxide solution, used as the neutralizing alkali liquid.
- Mixed catalyst (ratio of 3g K_2SO_4 to 0.2g $CuSO_4$) or commercially available catalyst tablets.
- 0.1mol/L hydrochloric acid or sulfuric acid standard titration solution.

Experimental Scheme and Operation Flow

Sample Pretreatment and Preparation

The goal of sample preparation is to improve the uniformity of the sample, so that it exhibits better repeatability and reproducibility in the experimental results.

1. **Rapid weighing:** Milk powder samples absorb moisture extremely easily in the air. Weighing should be done rapidly to avoid moisture absorption affecting the test results.

2. **Sampling:** Weigh approximately 0.5g (accurate to 0.1mg) of the fully mixed sample. Use weighing paper to wrap the sample, and place them together into the digestion tube.

3. **Reagent addition:** Add 2 mixed catalyst tablets (or 3.2g of mixed catalyst powder), and then add 10mL of concentrated sulfuric acid along the wall of the digestion tube.

4. **Sample blank:** To ensure the consistency of the digestion contents between the sample and the sample blank, a piece of weighing paper of the same specification as

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that used for the weighed sample must be added to the sample blank digestion tube and digested together.

Sample Digestion

The purpose of digestion is to destroy the original organic binding of nitrogen in the sample at high temperatures in the presence of sulfuric acid and a catalyst, converting all forms of nitrogen into ammonium ion form. For samples that are prone to moisture absorption and contain certain carbohydrates, it is recommended to set a curve heating program so that the digestion furnace heats the sample at a slow rate, keeping the foam generated by the sample in the initial stage within a controllable range.

Table 1: Graphite Digestion Furnace Parameter Settings

Stage	Temperature	Digestion Time
1	250℃	40min
2	420℃	120min

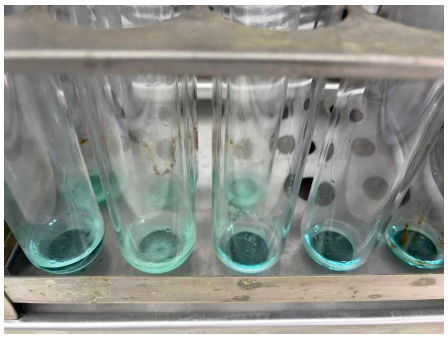


Figure 1: Solution after digestion completion

Instrumental Analysis

After digestion is complete, allow the digestion tubes to cool to room temperature before removing them for use. Check whether all components of the Kjeldahl nitrogen analyzer are functioning properly and whether reagents are sufficient. Perform an instrument blank test, and only proceed with sample analysis once the instrument blank reading is stable.

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Table 2: Nitrogen Analyzer Parameter Settings

Parameter	Set Value	Scientific Basis / Remarks
Boric Acid Volume	30 mL	Ensures complete complexation and absorption of distilled ammonia.
Dilution Water Volume	30 mL	Prevents violent acid-base neutralization and boiling upon alkali addition.
Alkali (NaOH) Volume	40 mL	The amount of strong base is typically 4 times the volume of sulfuric acid used for digestion, ensuring a strongly alkaline system to release ammonia.
Distillation Time	5 min	Guarantees complete mass transfer of ammonia from the liquid phase to the gas phase and into the condenser.
Titration Acid Concentration	0.0999mol/L	High-precision standard substance for quantitative titration of free alkali.
Protein Conversion Factor	6.38	Based on the general food-grade nitrogen-to-protein conversion ratio.

Experimental Data and Discussion

Representative hygroscopic milk powder samples were selected, and the experiment was conducted strictly following the above-mentioned pretreatment and instrument settings for digestion, distillation, and titration. The experimental results obtained are shown in the table below:

Table 3: Protein Content Test Results for Milk Powder Sample

Sample Name	Sample Weight (g)	Blank Volume (mL)	Titration Volume (mL)	Protein Content (%)	Average (%)	RSD/%
Milk Powder 1	0.9993	0.0621	5.0362	4.4381	4.4299	0.18
Milk Powder 2	1.0004	0.0621	5.0498	4.4215		
Milk Powder 3	0.9998	0.0621	5.0405	4.4302		

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Conclusion and Analysis

The average protein content of the milk powder sample tested using the fully automatic Kjeldahl nitrogen analyzer was 4.4299%, with an RSD value of 0.18%. The relative standard deviation is extremely low, indicating excellent repeatability of the experimental results. This demonstrates that by controlling the "rapid weighing" step for hygroscopic samples, combined with a programmed digestion ramp and fully automated Kjeldahl analysis, it is possible to effectively overcome moisture interference and foaming issues, achieving high-precision protein content detection.

Summary of Experimental Precautions

- **Strictly Prevent Hygroscopic Interference:** For samples prone to moisture absorption and caking, pay close attention to their moisture state. Ensure environmental humidity control and rapid weighing. If the sample has absorbed significant moisture, correct the final result by drying before weighing or separately determining the moisture content.
- **Ensure Representative Sampling:** Shake or mix the sample thoroughly before taking a subsample or weighing to ensure a uniform and representative state. This is a prerequisite for complete digestion.
- **Perform Matching Blank Tests:** Always reserve 1-2 sample blank tubes. Add the same catalyst, sulfuric acid, and auxiliary consumables (e.g., weighing paper) to the sample blank tubes as used for the test samples to deduct background errors from reagents and consumables.
- **Digestion Endpoint Determination:** The completion of digestion is indicated by a clear and transparent digest solution. If the sample is extremely difficult to digest, appropriately extend the holding time at 420 °C.

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