

Determination of Protein Content in Pure Milk Using Water-cooling-free Fully Automatic Kjeldahl Nitrogen Analyzer

Abstract

As a major source of high-quality protein in daily diet, accurate determination of protein content in pure milk is of great significance. In this experiment, samples were digested with a three-stage heating program. The Water-cooling-free Fully Automatic Kjeldahl Nitrogen Analyzer was used for precise measurement of protein content in pure milk samples. The results show that the average protein content of the samples is 3.38 %, and the relative standard deviation (RSD) is 0.32 %. This method features good repeatability and high precision.

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Introduction

Protein content is a core indicator for evaluating the nutritional value, quality grade and market compliance of pure milk, and is also a key controlled item in dairy food safety testing. This experiment was carried out in strict accordance with **GB 5009.5-2025 National Food Safety Standard — Determination of Protein in Food.** The classic Kjeldahl method was adopted, and its basic principle is as follows: under the action of a catalyst, organic matter is converted into ammonium sulfate by concentrated sulfuric acid at high temperature; then distillation is carried out under alkaline conditions, ammonia is released and absorbed by boric acid solution; finally, titration is performed with standard acid solution, and the total nitrogen content is calculated according to the consumption of acid, which is then converted into crude protein content. The principle of this method complies with international standards such as **ISO 8968-1:2014 and AOAC Official Method 991.20**.

Experimental Instruments and Reagents

Core Instruments

- Nitrogen analyzer: N810 Water-cooling-free Fully Automatic Kjeldahl Nitrogen Analyzer or N710 Fully Automatic Kjeldahl Nitrogen Analyzer
- Digestion equipment: NDS-20 Kjeldahl N-Digester
- Weighing equipment: Analytical balance with 1/10000 resolution (accurate to 0.1 mg)



Experimental Reagents

All reagents shall meet the requirements of analytical pure grade:

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- Basic reagents: Sulfuric acid (analytical pure), 20 g/L boric acid solution, 400 g/L sodium hydroxide solution
- Titration and indicator: 0.1 mol/L hydrochloric acid standard titration solution, bromocresol green-methyl red mixed indicator
- Catalyst: Mixed catalyst tablets, or self-prepared mixed catalyst powder (3 g K_2SO_4 and 0.2 g $CuSO_4$)
- Test sample: Boxed pure milk (protein content ≥ 3.3 g/100 mL), appearing as white liquid

Procedure

Sample Pretreatment and Preparation

1. **Sample weighing:** Use a disposable plastic dropper to draw approximately 2 g of pure milk sample (accurate to 0.1 mg) into the digestion tube by the weighing-by-difference method.
2. **Reagent addition:** Add 2 mixed catalyst tablets (or 3.2 g of mixed catalyst powder) into the tube, then slowly add 10 mL of concentrated sulfuric acid along the inner wall of the digestion tube.
3. **Blank preparation:** To ensure the consistency of the digestion system and subtract the reagent background, add the same amount of 2 mixed catalyst tablets and 10 mL of concentrated sulfuric acid into the reagent blank digestion tube, and digest it together with the samples.

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Figure 1. Samples before digestion

Heating Program

Place the digestion tubes containing samples, catalyst and reagents on the digester, cover the waste exhaust hood or place a curved-neck funnel at the opening of the digestion tube. Set three stages heating procedure, with specific parameters shown in Table 1.

Table 1. Digestion Parameter Settings

Stage	*Target Temperature*	*Time*
1	180°C	20min
2	250°C	30min
3	420°C	100min

Note: During the first heating stage (180 °C), the digestion tubes shall be heated without cover. The purpose is to evaporate a large amount of free water in the milk, concentrate and dry the liquid milk slowly, to avoid bumping or massive foaming during subsequent high-temperature heating.

Instrumental Analysis

After digestion is completed, remove the digestion tubes after cooling to room temperature for later use. Check whether all components of the nitrogen analyzer are in normal

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condition and whether reagents are sufficient. Meanwhile, perform instrument blank test, and start sample testing after the instrument blank become stable.

Table 2. Parameter Settings of Kjeldahl Nitrogen Analyzer

Parameter	*Value*
Boric acid volume	30 mL
Dilution water volume	30 mL
Alkali volume	40 mL
Distillation time	5 min
Titration acid concentration	0.1002mol/L
Protein conversion factor	6.38

Results and Discussion

Result

Representative pure milk samples were selected for digestion, distillation and titration in strict accordance with the above pretreatment and instrument settings. The experimental results are shown in Table 3.

Table 3. Test Results of Protein Content in Pure Milk Samples

Sample Name	*Sample Weight / g*	*Blank Volume / mL*	*Titration Volume / mL*	*Protein Content / %*	*Mean Value / %*	*RSD/%*
Sample-1	2.1209	0.0425	8.0762	3.3925	3.38	0.32
Sample-2	2.2077		8.3531	3.3715		
Sample-3	2.0063		7.6298	3.3870		

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Conclusion

For the pure milk samples tested by the Water-cooling-free Fully Automatic Kjeldahl Nitrogen Analyzer in this experiment, the mean protein content is 3.38 %, with an RSD value of 0.32 %. The relative standard deviation is at a very low level, indicating good repeatability of replicate determinations. This demonstrates that the combination of stepwise digestion procedure and fully automatic Kjeldahl nitrogen analysis can effectively overcome the problems of water bumping and foaming and achieve high-precision protein content detection.

References

[1] ISO 8968-1:2014, Milk and milk products — Determination of nitrogen content — Part 1: Kjeldahl principle and crude protein calculation.

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