

Determination of Protein Content in Wheat Flour Using Water-cooling-free Fully Automatic Kjeldahl Nitrogen Analyzer

Abstract

Protein content is a key indicator for evaluating the nutritional quality, processing performance and quality control of wheat flour. In accordance with the Kjeldahl method specified in GB 5009.5-2025 National Food Safety Standard — Determination of Protein in Food, this experiment determines the protein reference material in wheat flour with an N810 water-cooling-free fully automatic Kjeldahl nitrogen analyzer, and compares two digestion schemes: powdered catalyst and 15:1 catalyst tablet. The results show that both methods deliver results within the allowable range of reference material with satisfactory accuracy. The catalyst tablet method achieves an RSD of 0.20 %, featuring better precision and simpler operation, which provides data support for the optimization of laboratory detection methods.

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Introduction

Wheat flour is a staple grain product in the diet of Chinese residents. Protein content serves as a core indicator for the nutritional quality and processing suitability of wheat flour, and is also a critical item for grain quality regulation and pre-delivery inspection in food production.

This experiment is conducted in strict accordance with the first method (Kjeldahl method) of **GB 5009.5-2025** ***National Food Safety Standard — Determination of Protein in Food***. The water-cooling-free fully automatic Kjeldahl nitrogen analyzer is applied to determine the protein reference material in wheat flour. Meanwhile, the detection performance of two digestion schemes, the traditional powdered catalyst system and commercial catalyst tablets, are compared in terms of accuracy, precision and operational convenience, so as to provide data support for the optimization of routine laboratory detection protocols.



Instruments and Reagents

2.1 Instruments

N810 water-cooling-free fully automatic Kjeldahl nitrogen analyzer, NDS-20 Kjeldahl N-digester (20 positions), analytical balance with 0.1 mg readability.

2.2 Reagents

Sulfuric acid (analytical pure), 20 g/L boric acid solution, bromocresol green-methyl red mixed indicator, 400 g/L sodium hydroxide solution, potassium sulfate powder,

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copper sulfate pentahydrate powder, 15:1 mixed catalyst tablets, 0.1 mol/L hydrochloric acid standard titration solution.

2.3 Sample

Protein reference material (RM) in wheat flour (RM No. BWS0049-2016, Batch No. 2025110706). Certified value: 9.69 g/100g; characteristic value range: 8.91–10.47 g/100g; protein conversion factor: 5.70. (See Annex 1 for the reference material certificate.)



Experimental Procedure

3.1 Sample Preparation

Homogenize the wheat flour protein reference material. Accurately weigh approximately 0.5 g (accurate to 0.1 mg), wrap with weighing paper and transfer into a digestion tube. Prepare reagent blank simultaneously. Two digestion schemes are set as follows:

- **Method 1 (Powdered catalyst)**: Add 6 g of potassium sulfate and 0.4 g of copper sulfate pentahydrate, then slowly add 20 mL of concentrated sulfuric acid along the inner wall of the digestion tube.;
- **Method 2 (Catalyst tablet)**: Add 2 pieces of 15:1 mixed catalyst tablets, then slowly add 10 mL of concentrated sulfuric acid along the inner wall of the digestion tube.

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Note: To ensure consistent digestion matrix for samples and blanks, a piece of weighing paper is added to the blank digestion tube and digested together with samples.

3.2 Digestion

Place the digestion tubes loaded with samples, catalyst and reagents on the graphite digester. The parameter settings of the digester are listed in Table 1.

Table 1. Digestion Parameter Settings

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



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3.3 Instrumental Analysis

After digestion, cool the digestion tubes to room temperature before removal. Check the reagent level of the nitrogen analyzer. Run instrument blanks until three consecutive results are stable, then proceed to sample analysis. The operating parameters of the nitrogen analyzer for Method 1 and Method 2 are shown in Table 2 and Table 3 respectively.

Table 2. Operating Parameters for Method 1

Boric acid volume	*Dilution water volume*	*Alkali solution volume*	*Distillation time*	*Protein conversion factor*	*Titrant concentration*
30mL	30mL	80mL	5min	5.7	0.09998mol/L

Table 3. Operating Parameters for Method 2

Boric acid volume	*Dilution water volume*	*Alkali solution volume*	*Distillation time*	*Protein conversion factor*	*Titrant concentration*
30mL	30mL	40mL	5min	5.7	0.09998mol/L

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Results and Discussion

4.1 Experimental Results

All samples were processed through digestion, distillation and titration. The test results are summarized in Table 4.

Table 4. Protein Content Test Results

Sample	*Method*	*Sample Weight / g*	*Blank Volume / mL*	*Titration Volume / mL*	*Protein Content (g/100g)*	*Mean (g/100g)*	*Relative Deviation (%)*	*RSD (%)*
Protein quality control sample for wheat flour	Method 1(Powdered catalyst + 20 mL H_2SO_4)	0.5016	0.0248	6.1591	9.76	9.63	-0.62	1.13
		0.5006		6.0304	9.57			
		0.4995		6.0139	9.57			
	Method 2(Catalyst tablet + 10 mL H_2SO_4)	0.5000	0.0373	6.158	9.77	9.79	1.03	0.20
		0.4994		6.1664	9.79			
		0.4997		6.1789	9.81			

4.2 Conclusion

1. Accuracy: The mean protein contents obtained by the two schemes are 9.63 g/100g and 9.79 g/100g respectively, both falling within the characteristic value range of the reference material (8.91–10.47 g/100g). The relative deviations from the certified value (9.69 g/100g) are -0.62 % and 1.03 % respectively, well within the allowable error range of the method. This verifies that both catalytic digestion systems can guarantee the accuracy of detection

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results.

2. Repeatability: The relative standard deviation (RSD) of Method 1 is 1.13 %, while that of Method 2 is 0.20 %. The catalyst tablet method exhibits superior repeatability, which benefits from the uniform composition of tablet catalysts and high dosing consistency, eliminating operational errors caused by manual powder weighing.

3. Operation and cost: Method 2 only consumes 10 mL of concentrated sulfuric acid, a 50 % reduction compared with Method 1. In addition, catalysts are added directly in tablet form without separate weighing of potassium sulfate and copper sulfate, simplifying sample pretreatment.

References

[1] GB 5009.5-2025, National Food Safety Standard — Determination of Protein in Food[S].

[2] ISO 20483:2013, Cereals and pulses — Determination of the nitrogen content and calculation of the crude protein content — Kjeldahl method[S].

[3] AOAC Official Method 979.09, Protein in Grain, Kjeldahl Method[S].

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附件 1：

标准物质（RM）

标准物质编号：BWS0049-2016

标 准 物 质 证 书

Reference Material Certificate

小麦粉中蛋白质质控样品

Protein in wheat flour

批次编号 2025110706

Batch Number

定值日期 2025年11月07日

Certification Date

有效期至 2028年11月06日

Period of Validity

研制（生产）单位：北方伟业计量集团有限公司

Reference Material Producer

单位地址：河南省信阳市商城县城关镇何店轻工业园区

Address

联系电话：4000-999-322

Telephone

网址：www.bzwz.com

Website

版本号：A/0

Version

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本标准物质可用于粮食及粮食制品中蛋白质的相关分析检测，也可用于检测方法评价和仪器校准等实验室质量控制。

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本标准物质采用筛选合格的小麦粉为原料，蛋白质含量初测满足要求后，在洁净环境中，经过混匀、干燥、灭菌分装而成。

二、溯源性及定值方法

本标准物质以检测值作为标准值，方法参考《GB 5009.5-2025 食品安全国家标准 食品中蛋白质的测定》第一法凯氏定氮法。通过使用满足计量学特性要求的制备、测量方法和计量器具，保证标准物质量值的溯源性。

三、特征量值及扩展不确定度

组分	CAS	标准值	扩展不确定度 (k=2)	基体
蛋白质	/	9.69 g/100g	0.78 g/100g	小麦粉

标准值的扩展不确定度主要由定值、均匀性及稳定性等不确定度合成。

四、均匀性检验及稳定性考察

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