

Evaluation of Sulfuric Acid Pretreatment Time for Protein Determination in Soybean Curd-Skin Rolls Using a Water-Cooling-Free Fully Automatic Kjeldahl Nitrogen Analyzer

Abstract

Protein content is an important compositional parameter for soybean-based food products and is commonly determined by the Kjeldahl method. This application note evaluates the effect of sulfuric acid pretreatment time on protein determination in soybean curd-skin rolls using the N810 Water-Cooling-Free Fully Automatic Kjeldahl Nitrogen Analyzer. Three sulfuric acid pretreatment times, namely 0, 1 and 2 h, were evaluated under otherwise consistent digestion and instrumental conditions. The mean protein contents obtained were 21.58, 21.65 and 21.33 g/100 g, respectively, with relative standard deviation (RSD) of 0.10%, 1.05% and 0.30%. No clear systematic effect of sulfuric acid pretreatment time on the measured protein content was observed within the tested range.

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1. Introduction

Protein content is an important quality parameter of soybean-based food products. The Kjeldahl method is an established reference approach for determining nitrogen content, from which crude protein content can be calculated using an appropriate protein conversion factor. ISO 1871:2009 provides general guidelines for Kjeldahl nitrogen determination in food and feed products, while GB 5009.5-2025 specifies the determination of protein in foods and gives a conversion factor of 6.25 for soybean and soybean products.

Soybean curd-skin products such as “Xiangling rolls” are solid, protein-rich food matrices. During Kjeldahl analysis, the addition of concentrated sulfuric acid initiates dehydration and acid digestion of the sample matrix. The contact time between the sample and sulfuric acid before catalyst addition may affect the initial digestion behavior and sample condition. This application note evaluates different sulfuric acid pretreatment times using the N810 Water-Cooling-Free Fully Automatic Kjeldahl Nitrogen Analyzer to determine whether extended acid pretreatment provides a measurable benefit under the tested conditions.

2. 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

Unless otherwise specified, reagents were of analytical grade or equivalent.

Sulfuric acid (analytical pure), 20 g/L boric acid solution, bromocresol green-methyl red mixed indicator, 400 g/L sodium hydroxide solution, 15:1 mixed catalyst tablets, 0.1 mol/L hydrochloric acid

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standard titration solution.

3. Experimental Procedure

3.1 Sample Preparation

The "Xiangling roll" sample was pulverized and thoroughly homogenized before analysis. Approximately 0.3 g of sample was accurately weighed to 0.1 mg, wrapped with weighing paper, and transferred into a digestion tube. Reagent blanks were prepared simultaneously using the same weighing paper and digestion reagents as the sample tubes. Duplicate determinations were performed for the 0 h condition, while triplicate determinations were performed for the 1 h and 2 h conditions.

3.2 Sulfuric Acid Pretreatment

After sample weighing, 15 mL of concentrated sulfuric acid was slowly added along the inner wall of each digestion tube. The sample and sulfuric acid were mixed thoroughly and allowed to stand for different pretreatment periods before catalyst addition.

Three pretreatment conditions were evaluated: immediate digestion after sulfuric acid addition (0 h), 1 h sulfuric acid pretreatment, and 2 h sulfuric acid pretreatment. After the designated pretreatment period, three 15:1 mixed catalyst tablets were added to each digestion tube, and the tubes were transferred to the digester for digestion.

3.3 Digestion

The digestion program was kept identical for all pretreatment conditions to ensure that the effect of sulfuric acid pretreatment time could be evaluated under consistent digestion conditions. The parameter settings of the digester are listed in Table 1.

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Table 1. Digestion Parameter Settings

Stage	Temperature	Time
1	180℃	20min
2	250℃	40min
3	420℃	60min

Note: In stage 1 and 2, the digestion tubes should be heated without any cover. The waste exhaust hood or bent-neck funnel will only be installed after stage 2 completed.

3.4 Instrumental Analysis

After digestion, the digestion tubes were cooled to room temperature before instrumental analysis. Check whether sufficient reagents are available in the nitrogen analyzer. Run instrument blanks until three consecutive results are stable, then proceed to sample analysis. The N810 operating parameters were set as table 2.

Table 2. Operating Parameters

Boric acid volume	*Dilution water volume*	*Alkali solution volume*	*Distillation time*	*Protein conversion factor*	*Titrant concentration*
30 mL	30 mL	60 mL	5 min	6.25	0.1002 mol/L

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4. Results and Discussion

Experimental Results

The protein contents obtained under the three sulfuric acid pretreatment conditions are summarized below.

Table 3. Test Results of Protein Content

Sulfuric Acid Pretreatment Time	*Sample Weight / g*	*Blank Volume / mL*	*Titration Volume / mL*	*Protein Content (g/100g)*	*Mean (g/100g)*	*RSD/%*
0h	0.3056	0.0767	7.5928	21.56	21.58	0.10
	0.3010		7.4896	21.59		
1h	0.3023		7.6016	21.82	21.65	1.05
	0.3046		7.6306	21.74		
	0.3036		7.4855	21.40		
2h	0.3018		7.4393	21.39	21.33	0.30
	0.3015		7.389	21.26		
	0.3028		7.4476	21.34		

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Discussion

The mean protein content obtained after 0, 1 and 2 h of sulfuric acid pretreatment were 21.58, 21.65 and 21.33 g/100 g, respectively, with RSD values of 0.10%, 1.05% and 0.30%. The difference between the highest and lowest mean values was 0.32 g/100 g, and no clear monotonic trend was observed with increasing pretreatment time.

5. Conclusion

1. The Kjeldahl procedure provided consistent protein determination for the soybean curd-skin roll sample evaluated in this study.
2. No clear systematic effect of sulfuric acid pretreatment time on the measured protein content was observed within the tested range of 0–2 h.
3. Under the evaluated conditions, immediate digestion after sulfuric acid addition provided a protein determination result comparable to those obtained after 1 h and 2 h of pretreatment. Therefore, extended sulfuric acid pretreatment did not provide a clear analytical benefit for the tested “Xiangling roll” sample and digestion procedure.

References

1. GB 5009.5-2025, National Food Safety Standard — Determination of Protein in Food.
2. ISO 1871:2009. Food and feed products — General guidelines for the determination of nitrogen by the Kjeldahl method.
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