APPLICATION NOTE

F&F-K-001-2015/A1

N/Protein Determination in Soybean

according to the Kjeldahl method

Reference: **AOAC 920.87 (945.39 and 979.09) Protein (Total) in Flour**

Tested with **VELP Scientifica DKL 20 Automatic Kjeldahl Digestion Unit** (Code S30100210) and **UDK 169 Automatic Kjeldahl Analyzer with AutoKjel Autosampler** (Code S30200160)

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N/PROTEIN DETERMINATION IN SOYBEAN
KJELDAHL METHOD

Introduction

Soybean meal is the most important protein source used to feed farm animals. It represents two-thirds of the total world output of protein feedstuffs, including all other major oil meals and fish meal (Oil World, 2010). Indeed it is an important part of the diets of ruminants animals due to its high amount (more than 60%) of rumen-degradable protein, good amino acid balance and high cell-wall digestibility, besides being also very palatable to ruminants.

Soybean meal is the by-product of the extraction of soybean oil. In the solvent extraction process, the soybeans are cracked, heated, flaked and the oil is extracted by solvent. The extracted flakes are then dried to eliminate the solvent, toasted and ground. The soybeans may have been de-hulled prior to extraction, and the hulls may be added back at the end of the process.

Nitrogen Determination in Soybean Powders according to the Kjeldahl Method

Thanks to the high level of precision and reproducibility and to its simple application, Kjeldahl is nowadays the most used method for determining nitrogen and protein contents in the food and feed industry. It also has several other applications in environmental control (phenols and nitrogen in water, sludge, soil and lubricants) and in the chemical and pharmaceutical industry according to official AOAC, EPA, DIN e ISO procedures.
The modern Kjeldahl method consists in a procedure of catalytically supported mineralization of organic material in a boiling mixture of sulfuric acid and sulfate salt at digestion temperature higher than 400 °C. During the process the organically bonded nitrogen is converted into ammonium sulfate. Alkalizing the digested solution liberates ammonia which is quantitatively steam distilled and determined by titration.

Samples

Soybean powder A	Expected Protein Value: > 70 %
Soybean powder B	Expected Protein Value: > 80 %
Soybean powder C	Expected Protein Value: > 80 %
Soybean powder D	Expected Protein Value: > 50 %

Sample Digestion

Samples are already homogenized (particle size 0.5 mm).
Weigh about 0.300 g of sample into a nitrogen-free weighing boat (code CM0486000) and place it into a 250 ml test tube. For each sample, add in the test tube:

- 2 catalyst tablet VCM (code A00000274; 3.5 g K_2SO_4, 0.1 g Cu)
- 15 ml concentrate sulphuric acid (96-98%)

Prepare some blanks with all chemicals and without the sample.
Connect the Digestion Unit to a proper Aspiration Pump (JP code F30620198) and a Fume Neutralization System (SMS Scrubber code F307C0199) to neutralize the acid fumes created during digestion phase.
Digest the samples for 90 minutes at 420 °C, according to the method “soya beans and lupins” (n° 10 on DKL 20).

Distillation and Titration

Let the test tubes cool down to 50-60 °C.
Condition the **UDK 169 with AutoKjel Autosampler** unit by performing the Automatic Check-up and Wash-down in the Menu-System.

Distill the samples according to the following parameters (pre-defined method n° 10):

- H_2O (dilution water): 70 ml
- NaOH (32 %): 55 ml
- H_3BO_3 (4 % with indicators): 30 ml
- H_2SO_4 (0.1 N) as titrant solution
- Protein factor: 6.25

Distillation & Titration analysis time: from 4 minutes for one test.

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Typical Results on Soybean Powders

The results are automatically calculated by UDK 169 as percentage of nitrogen and percentage of protein.

Sample	Sample quantity (g)	Nitrogen %	Protein %
Soybean powder A	0.296	12.063	75,394
	0.305	12.169	76,057
	0.305	12.154	75,962
	0.304	12.123	75,769
	Average ± SD%	12.127 ± 0.047	75.795 ± 0.293
	RSD% *	0.387	0.387
Soybean powder B	0.307	13.257	82,859
	0.304	13.332	83,328
	0.306	13.226	82,663
	0.297	13.189	82,433
	Average ± SD%	13.251 ± 0.061	82.821 ± 0.380
	RSD% *	0.459	0.459
Soybean powder C	0.302	13.253	82,833
	0.300	13.206	82,539
	0.301	13.426	83,911
	0.301	13.204	82,524
	Average ± SD%	13.272 ± 0.105	82.952 ± 0.655
	RSD% *	0.790	0.790
Soybean powder D	0.303	8.564	53.523
	0.303	8.390	52.437
	0.294	8.556	53.476
	0.307	8.454	52.837
	Average ± SD%	8.491 ± 0.084	53.068 ± 0.525
	RSD% *	0.988	0.988
Protein Factor: 6.25			

***** RSD% = (Standard Deviation * 100) / Average

The complete procedure was verified by using 5 ml of glycine standard solution (3%) containing 28 mg of nitrogen. as reference substance. The obtained recovery falls into the expected range: between 98 % and 102 %.

Conclusions

The obtained results are reliable and reproducible in accordance with the expected values: all data fulfill the expected range.

Benefits of Kjeldahl method by using DKL 20 and UDK 169 with AutoKjel Autosampler are:

- High level of precision and reproducibility
- Maximum productivity and full automation
- Worldwide official method
- Reliable and easy method
- Time saving
- Moderate running costs

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