APPLICATION NOTE

F&F-K-009-2013/A2

N/Protein Determination in Cereals

according to the Kjeldahl method

Reference: **AOAC 979.09** Protein in Grains; **AACC 46.11 A**

Tested with **VELP Scientifica DKL 20 Automatic Kjeldahl Digestion Unit** (Code S30100210) and **UDK 159 Automatic Kjeldahl Distillation & Titration System** (Code F30200150)

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N/PROTEIN DETERMINATION IN CEREALS KJELDAHL METHOD

Introduction

Cereals contain between 8-15% of different kinds of proteins such as albumins, globulins, prolamines, gliadins, glutelins and glutenins. Their chemical composition is important not only for nutritional purpose, but also for the bread dough and its process of baking. Gliadins and glutenins, with water contact, form *gluten*, a lipoproteic substance that gives viscosity, elasticity and cohesion to dough, helping it to rise and to keep its shape; it is found in wheat and other grains, including barley and rye.
There is currently a large interest in *gluten* for its technological application, but also for its health concerns (coeliac disease). *Gluten* is not naturally occurring in corn, rice, or oats, but may be cross-contaminated by facilities that also process wheat, barley, or rye products.
For legal purpose it's important to know the proteins amount in cereal flours, because, generally, their commercial quality depends on it.

Protein Determination in cereals according to the Kjeldahl method

Kjeldahl is nowadays the most used method for determining nitrogen and protein contents in foods and feeds thanks to the high level of precision and reproducibility and to its simple application.
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 temperatures 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.

Sample

Cereals Wheat: protein expected value 14.2% Corn: protein expected value 8.0%

Sample Digestion

Grind finely the sample using a cereals mill (particle size 0,5 mm).
Weight about 2 grams in a nitrogen-free weighing boat (Code CM0486000) and transfer in a test tube.
In each the test tube add:

- 2 catalyst tablets VCM (code A00000274; 3.5 g K_2SO_4, 0.1 g $CuSO_4$ $5H_20$ Missouri)
- 2 antifoam tablets VS (Code A00000283)
- 20 ml concentrated sulphuric acid (96-98%)

Prepare some blanks with all chemicals and without 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 sample for 40 minutes at 300 °C plus 90 minutes at 420 °C, according to the method "Wheat" (n°8 on DKL 20).

Distillation and Titration

Let the test tubes cool down to 50-60 °C.
Condition the UDK 159 unit by performing the Automatic Check up in Menu-System and a Wash down.
Distill the samples selecting the predefined methods n° 8 (for wheat samples) and n°9 (for other cereals samples).

- H_2O (dilution water): 50 ml
- H_2SO_4 (0.2 N) as titrant solution
- NaOH (32%): 70 ml
- Protein factor: 5.70 (wheat) and 6.25 (other cereals)
- H_3BO_3 (4% with indicators): 30 ml

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

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Typical Results on Cereals

The results are calculated as a percentage of nitrogen and percentage of protein.

Sample	Sample quantity (g)	Nitrogen %	Protein %
Durum Wheat	2.014	2.510	14.308
	2.000	2.498	14.238
	2.007	2.498	14.237
	2.006	2.525	14.395
	2.001	2.520	14.365
	Average ± SD%	2.510 ± 0.012	14.309 ± 0.072
	RSD% *	0.494	0.503
Yellow Corn	2.106	1.260	7.877
	2.004	1.271	7.943
	2.000	1.279	7.997
	2.003	1.289	8.057
	2.028	1.268	7.928
	Average ± SD%	1.274 ± 0.011	7.960 ± 0.069
	RSD% *	0.863	0.865
Protein Expected Value: 14.2% for Wheat; 8.0% for Corn			
Protein Factor: 5.70 for Wheat; 6.25 for Corn			

* RSD% = (Standard Deviation x 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%.

Conclusion

The obtained results are reliable and reproducible in accordance with the expected values, with a low relative standard deviation (RSD < 1%), that means high repeatability of the results.

Benefits of Kjeldahl method by using DKL 20 and UDK 159 are:

- High level of precision and reproducibility
- High productivity
- Worldwide official method
- Reliable and easy method
- Time saving
- Affordable equipment cost
- Moderate running costs

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