APPLICATION NOTE

F&F-D-003-2017/A1

N/Protein Determination in Barley

Dumas and Kjeldahl method comparison

Kjeldahl reference: **AOAC 979.09** Protein in Grains; **AACC 46.11 A, ASBC Barley 7A**

Dumas reference: **AOAC 992.23** Crude Protein in Cereal Grains and Oilseeds, **AACC 46-30.01**, **ASBC Barley 7C**

Tested with **VELP Scientifica DKL 20 Automatic Kjeldahl Digestion Unit** (Code S30100210)
UDK 169 Automatic Kjeldahl Analyzer with AutoKjel Autosampler (Code S30200160) and
VELP Scientifica NDA 702 Dual Carrier Gas Dumas Nitrogen Analyzer (Code F30800080)

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N/PROTEIN DETERMINATION IN BARLEY
DUMAS AND KJELDAHL METHOD COMPARISON

Introduction

Protein content of malting barley is important because it affects malting, brewing, and their end products. Kjeldahl determination is the commonly accepted method of protein estimation in barley, based on traditional wet chemistry.
An alternative to the classical Kjeldahl method is the Dumas combustion technique, innovative dry chemistry, easy to use and highly accurate.
Both the techniques are officially approved for the determination of the nitrogen and protein content in barley.

Protein Determination in GAFTA Barley

This application note compares the nitrogen/protein determination in barley by using **NDA 702 Dumas Nitrogen Analyzer** and **UDK169 Automatic Kjeldahl Analyzer with AutoKjel Autosampler**.

The specific methods used in this study are summarized briefly here:

Kjeldahl method

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.

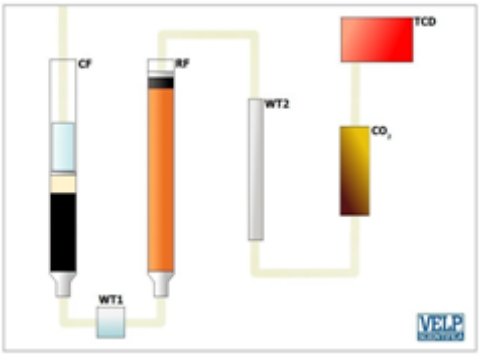
Dumas method

The Dumas method starts with a combustion furnace (CF) to burn the sample, obtaining elemental compounds.

Water is removed by a first physical trap (WT1 - **DriStep™**), placed after the combustion, and a second chemical one (WT2). Between the two, the elemental substances passed through a reduction furnace (RF).

The auto-regenerative CO_2 absorbers (CO_2) let pass only the elemental nitrogen that is detected by the **LoGas™** innovative Thermal Conductivity Detector (TCD) with no requirement for a reference gas.

The NDA 702 is controlled via PC through the intuitive **DUMASoft™**.



Performances of VELP Kjeldahl system and Dumas unit were evaluated by determining the protein content of a GAFTA (The Grain and Feed Trade Association) reference sample, tested with both techniques by over 250 laboratories, in 53 countries.

The obtained results (**as % Protein**) were compared with the **GAFTA assigned value**.

Sample

Un-milled barley

Sample ID: 2016:G2

Protein assigned value: 7.560 ± 0.282 g/100g

The sample has been grinded by using a laboratory cereal mill (particle size 1mm).

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Kjeldahl analysis

1. Sample Digestion

Weigh about 2 grams in a nitrogen-free weighing boat (Code CM0486000) and transfer in a test tube.

In each 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 sulfuric 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 “Oats, barley, corn, rice, rye” (n°9 on DKL 20).

2. Distillation and Titration

Let the test tubes cool down to 50-60 °C.

Condition the UDK 169 unit by performing the Automatic Check up in Menu-System and a Wash down.

Distill the samples selecting the predefined methods n°9.

- H_2O (dilution water): 50 ml
- H_2SO_4 (0.2 N) as titrant solution
- NaOH (32%): 70 ml
- Protein factor: 6.25
- Vreceiver™ (A00000316): 30 ml

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

Dumas analysis

1. NDA 702 Preliminary Operations (daily)

Follow the operating manual to start the NDA 702 and check that the following parameters are set:

Temperature Combustion reactor (Code A00000158): 1030 °C

Temperature Reduction reactor (Code A00000226): 650 °C

Flow rate MFC1 He: 190 ml/min

Flow rate MFC2 He: 220 ml/min

Condition the system by testing 2 EDTA standard (Code A00000149) and 3 to 5 empty tin foils (Code A00000153) as Check up. Verify the calibration curve with one or more tests as Standard by testing the same standard used for the curve creation.

2. Sample Preparation (NDA 702)

Weigh around 200 mg of sample in a tin foil directly on the analytical balance.

Close the tin foil, obtaining a capsule.

Load the capsule into the autosampler.

3. Analysis Procedure (NDA 702)

Fill the following fields in the database of the software Dumasoft™: **Sample name, Weight, Method, Sample type, Calibration number**

The “CEREAL MEAL 1” method shows the following parameters:

Protein factor: 6.25 | **O_2 flow rate**: 400 ml/min | **O_2 factor**: 1.6 ml/mg

Press  to start the analysis. Analysis time: from 3 minutes for one run.

Results have been obtained with the following calibration curve: in a range of 0 - 7 mg N with 7 measurements of EDTA standard (%N = 9.57) (Code A00000149).

The data obtained are included in the tolerance admitted by the EDTA certificate.

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Results on GAFTA Barley standard

The table below shows the nitrogen/protein results, obtained by **NDA 702 Dumas analyzer** and **VELP Kjeldahl systems DKL 20** and **UDK 169**:

Technique	Sample quantity (mg)	Nitrogen %	Protein %
Dumas	199.10	1.237	7.732
	204.30	1.228	7.677
	199.40	1.229	7.680
	205.80	1.233	7.703
	203.70	1.212	7.573
	Average ± SD%	**1.228 ± 0.010**	**7.673 ± 0.060**
	RSD% *	**0.776**	**0.783**
Kjeldahl	2019	1.197	7.480
	2009	1.205	7.533
	2009	1.206	7.539
	1967	1.199	7.493
	2069	1.202	7.512
	Average ± SD%	**1.202 ± 0.004**	**7.511 ± 0.025**
	RSD% *	**0.339**	**0.339**
Expected Protein Value: 7.560 ± 0.282 g/100g			
Protein Factor: 6.25			

Conclusions

The obtained values fell within the expected protein range of the standard material certified by GAFTA, demonstrating the high performances of both type of VELP equipment, Kjeldahl system and Dumas analyzer NDA 702.

Excellent repeatability is ensured with both techniques, as demonstrated by low RSD values.

VELP Kjeldahl modern system, using genuine catalyst tablets KJTabs™, is still a robust solution for protein determination in food and feed field.

NDA 702 Dumas combustion apparatus with high productivity and non-stop performance is indeed ideal for high throughput, being fully automated and requiring just 3-4 minutes per analysis.

In conclusion, both techniques are efficient and capable of analyzing the barley sample with high accuracy and repeatability.

VELP offers a wide range of solutions for protein determination in food and feed. Just find the best alternative according to the laboratory requirements.

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