Application Note: 42122

Nitrogen/Protein determination in Cereals and Seeds by the Thermo Scientific FLASH 4000 Series

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Key Words

- Cereals
- Flash Combustion
- Food Safety
- Nitrogen/Protein
- Seeds

Introduction

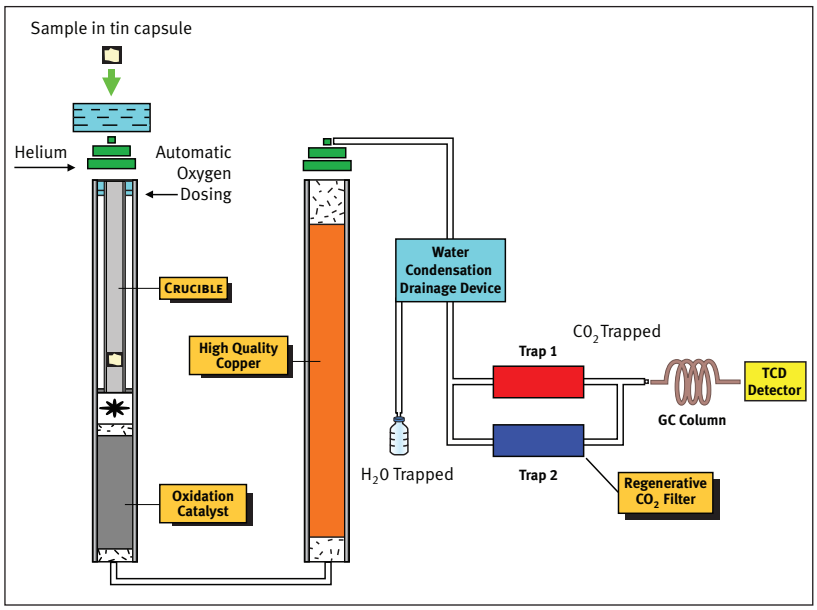
Cereals and seeds are significant components of the human diet and the principal part of feeding stock for domestic animals. One of the most important nutrients is Protein and the monitoring of its amount, through the determination of Nitrogen, must be accurate to determine the nutritional quality of these products. In addition to its dietary importance, the Protein content also has become a guideline for some cereal trade transactions.

For this reason, the capabilities of the combustion method have been greatly improved to make it faster, safer and more reliable than the traditional Kjeldahl method.

As a direct consequence of these advantages, the combustion method was approved and adopted by the Association of Official Analytical Chemists (AOAC method 990.03) and American Association of Cereal Chemists (AACC method 46-30).

The FLASH 4000 N/Protein Analyzer, based on the dynamic flash combustion of the sample, copes effortlessly with the wide array of laboratory requirements such as accuracy, day-to-day reproducibility and high sample throughput.

Analytical Configuration



Analytical conditions

Temperature left reactor: 950 °C
Temperature right reactor: 840 °C
Temperature oven: 50 °C

Carrier Flow (He): 300 ml/min
Reference Flow (He): 300 ml/min

Standard: 500 mg EDTA (9.59 %N)

Sample weight: 600 mg - 1.6 g

Note: The Oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune® function present in the Eager Xperience dedicated software.

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Official methodology requirements for Protein determination by Combustion

AOAC (Association of Official Analytical Chemists, Method 992.23) and AACC (American Association of Cereal Chemists, Method 46-30, 1999) indicate that the suitable fineness of grind must be determined (for each different material analyzed) to achieve precision that gives RSD of ≤ 2 % for 10 successive determinations of Nitrogen.

Results

Different cereals and seeds were chosen to validate the system in terms of accuracy and reproducibility according with the pre-treatment of the sample. The Protein content is calculated automatically by the Thermo Scientific Eager Xperience Software using the default Protein factor of 6.25 (5.70 for rice). The Protein factor can be changed in accordance with the food type.

Table 1 shows the Nitrogen and Protein determination in **cereals** samples. **Barley** and **rice** were homogenized to particle size 1 mm and 2 mm. The data obtained of 10 consecutive determinations show an excellent reproducibility. In all cases the relative standard deviation was less than 2 %, according to the official methods. No memory effect was observed when changing the type of sample, indicating the complete detection of the Nitrogen present in the sample. No significant differences in the results were observed changing the sample particle size from 2 to 1 mm.

Table 1 – N/Protein determination in cereals

RICE						BARLEY					
Particle size 1 mm			Particle size 2 mm			Particle size 1 mm			Particle size 2 mm		
Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %
1004.9	1.2105	6.8998	997.2	1.1998	6.8387	997.1	1.9385	12.1159	987.4	1.9260	12.0375
996.3	1.1913	6.7902	1009.2	1.2105	6.9001	998.4	1.9279	12.0494	1003.9	1.9465	12.1657
996.2	1.2010	6.8460	1011.4	1.2264	6.9904	999.8	1.9106	11.9411	990.0	1.9256	12.0348
1005.3	1.2032	6.8580	999.8	1.2092	6.8925	1002.6	1.9483	12.1772	1004.9	1.8954	11.8464
1005.2	1.2116	6.9058	994.8	1.1822	6.7383	1004.9	1.9449	12.1554	1006.2	1.9427	12.1420
1006.1	1.2051	6.8692	1006.0	1.1966	6.8205	995.2	1.9651	12.2820	993.3	1.9027	11.8920
1007.9	1.2017	6.8496	1004.7	1.1979	6.8283	992.4	1.9563	12.2267	998.6	1.9298	12.0611
1001.3	1.1972	6.8240	992.7	1.1801	6.7265	995.3	1.9564	12.2276	1005.1	1.9190	11.9937
1001.3	1.2019	6.8506	1009.1	1.1895	6.7800	1003.0	1.9648	12.2797	1010.4	1.9153	11.9708
1009.5	1.1942	6.8070	1009.2	1.2142	6.9211	992.5	1.9541	12.2134	1006.6	1.8944	11.8398
Av.N %	**1.2017**	**6.8500**	**Av.N %**	**1.2006**	**6.8437**	**Av.N %**	**1.9467**	**12.1669**	**Av.N %**	**1.9197**	**11.9983**
RSD %	**0.5362**	**0.5362**	**RSD %**	**1.220**	**1.220**	**RSD %**	**0.879**	**0.879**	**RSD %**	**0.945**	**0.945**

Table 2 shows the Nitrogen and Protein determination in **sunflower seeds**. Oil-seeds due to the sample nature, high content of fat, require a proper optimization of the Oxygen amount needed for combustion to obtain accurate data. The sample was homogenized at 2 mm particle size. The data are reproducible with a RSD % as per official method requirements. The left part shows the reproducibility of 10 determinations using a weight of around 500 mg, while in the right part indicates the reproducibility of 10 determinations in a range from 700 to 1000 mg of the same sunflower sample. No memory effect was observed when changing sample weight.

Table 2 – N/Protein determination in sunflower seeds

Weight range 500 mg			Weight range 700-1000 mg		
Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %
501.1	3.0124	18.8276	998.1	3.0884	19.3022
499.2	2.9752	18.5949	694.9	3.0924	19.3276
495.4	3.0037	18.7729	991.5	3.0413	19.0084
497.5	3.0014	18.7588	795.1	3.0288	18.9299
505.0	3.0693	19.1832	692.2	3.0745	19.2156
497.5	3.0648	19.1550	994.9	3.0523	19.0773
497.9	3.0308	18.9427	802.4	3.0658	19.1612
505.9	3.0542	19.0887	996.5	3.0773	19.2331
495.3	3.0336	18.9602	897.6	3.0607	19.1295
486.7	3.0529	19.0804	1002.1	3.0584	19.1150
Av.N %	**3.0298**	**18.93647**	**Av.N %**	**3.0640**	**19.1500**
RSD %	**1.0263**	**1.0263**	**RSD %**	**0.6546**	**0.6546**

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