Nitrogen/Protein Determination in Starch by Flash Combustion using Large Sample Weight as an Alternative to the Kjeldahl Method

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Overview

Purpose: To show the determination of Nitrogen / Protein in starch samples by flash combustion at high sample weight.

Methods: Starch samples were analyzed using an elemental analyzer with an automatic autosampler.

Results: Data collected of Nitrogen / Protein from different starch samples are discussed to assess the performance of the analyzer.

Introduction

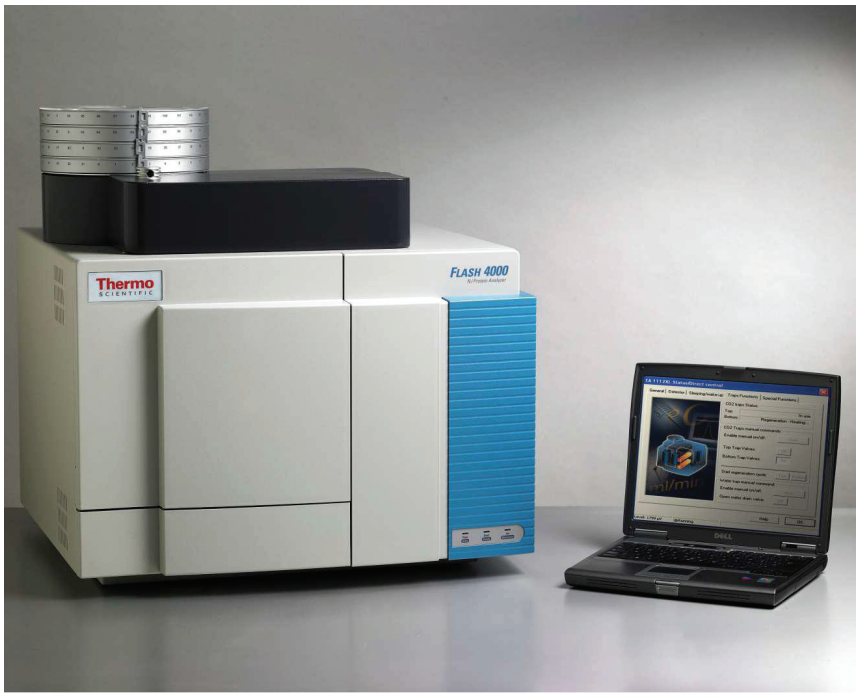
The production process in the starch industry, the protein content, calculated by the determination of nitrogen, is periodically monitored and tested for quality control. Because starch is used in the preparation of animal feeds, bakery products, puddings, instant meals, syrups and desserts, the determination of N/Protein is critical.

It is therefore very important to have a method which allows the fast analysis of N/Protein with an excellent reproducibility.

The Thermo Scientific™ FLASH 4000 Nitrogen/Protein Analyzer (Figure 1), based on the dynamic flash combustion of the sample, satisfies all the requirements of modern laboratories such as stability, accuracy, day-by-day reproducibility and high sample throughput , and does not require sample digestion or toxic materials. This alternative to the classical Kjeldahl method, based on Dumas (combustion) method, has been developed and approved by different associations such as ASBC, AOAC, AACC, AOCS, IFFO and ISO.

This paper presents data on Nitrogen/Protein determination of different starch samples in a large range of concentrations (150 – 2500 ppm nitrogen), obtained with the analyzer using large sample weight to demonstrate the validity of the method without matrix effect. Data compared to the results obtained by the Kjeldahl method demonstrates the validity of the system.

FIGURE 1. FLASH 4000 Nitrogen / Protein Analyzer.



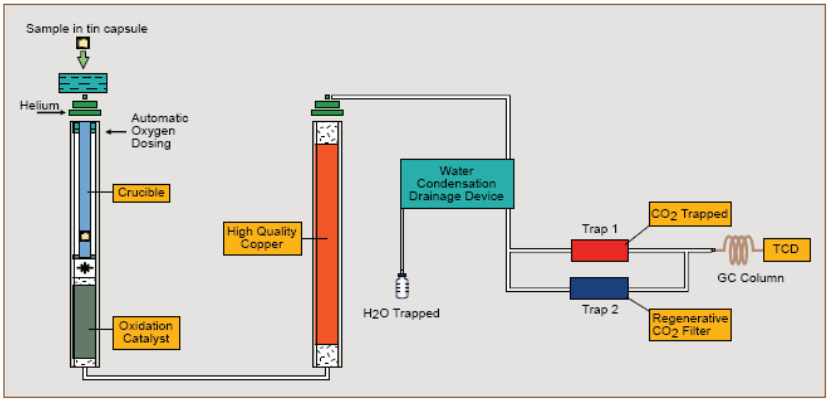
Method

The sample is weighed in a tin capsule and introduced into the combustion reactor via the Thermo Scientific™ MAS™ 4000 autosampler together with the correct amount of oxygen using the Thermo Scientific™ OxyTune™ function, insuring a complete combustion of the sample. The samples are weighed directly in the tin capsule in the range of 1000 - 2000 mg.

After combustion, the produced gases are carried by a helium flow to a second reactor filled with copper. Water is trapped through a Peltier system while the CO_2 is adsorbed by the No-Stop Twin traps. Then the nitrogen is swept through a GC column and finally is detected by a thermal conductivity detector (TCD) (Figure 2).

A complete report is automatically generated by the Thermo Scientific™ Eager Xperience dedicated data handling software, and is displayed at the end of the analysis. Eager Xperience also allows the direct sample weight transfer from the balance to the sample table, and the complete control of the analytical parameters of the instrument.

FIGURE 2. Nitrogen / Protein configuration



Analytical conditions:

Left Furnace Temperature :	950°C
Right Furnace Temperature :	840°C
Oven Temperature:	50°C
Carrier Flow:	300 ml/min
Reference Flow:	300 ml/min
Standard:	500 mg EDTA* (9.59 %N)
Sample Weight:	1000 - 2000 mg

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

*EDTA: EthyleneDiamineTetraAcetic acid

Results

The starch samples analyzed were chosen on basis of their differing nitrogen content. The data obtained demonstrates the no-matrix effect in the determination of nitrogen, indicating complete combustion for all type of samples.

The calibration of the FLASH 4000 was performed with EDTA (9.59 %N) using K factor as calibration method.

Table 1 shows the nitrogen results obtained of a starch sample of approximately 0.25 % N, analyzed ten times at about 1 gram. Table 2 shows the nitrogen data obtained analyzing the same starch in a range from 700 mg to 2 grams. In both cases the data are comparable and no significant difference was observed changing the sample weight.

TABLE 1. Nitrogen data of starch (0.25 %N) at 1 gram.

Sample weight (mg)	N %	Average N %	RSD %
981.0	0.2547	0.2527	1.0520
997.4	0.2500		
984.8	0.2518		
998.3	0.2550		
1032.2	0.2529		
1013.4	0.2514		
998.0	0.2579		
1017.3	0.2502		
964.3	0.2496		
987.6	0.2538		

TABLE 2. Nitrogen data of starch (0.25 %N) in the range of 700 mg to 2 grams.

Sample weight (mg)	N %	Average N %	RSD %
797.7	0.2536	0.2556	1.4550
946.2	0.2530		
1101.6	0.2528		
1255.0	0.2600		
1401.4	0.2556		
1611.1	0.2571		
2001.5	0.2637		
987.7	0.2522		
802.7	0.2536		
1025.0	0.2541		

Table 3 shows the Nitrogen / Protein data of a starch sample of approximately 400 ppm N weighed in a range of 1000 – 1100 mg.
The protein content was calculated automatically by the Eager Xperience dedicated software using 6.25 as protein factor.

TABLE 3. Nitrogen / Protein data of starch (0.04 %N) in the range of 1.0 – 1.1 grams.

N%	RSD %	Protein %	RSD % 2
0.0472		0.2947	
0.0484		0.2756	
0.0430		0.2736	
0.0454		0.2640	
0.0427	5.0777	0.2636	5.0535
0.0419		0.3022	
0.0441		0.2685	
0.0438		0.2841	
0.0422		0.2667	
0.0422		0.2619	

Table 4 shows the sequence of analysis of starch samples in trace level analyzed randomly to evaluate memory effect when changing the sample type and nitrogen content.

Table 5 shows the statistical data of the relative samples. The protein content was calculated automatically by the Eager Xperience dedicated software using 6.25 as protein factor.
No memory effect was observed indicating complete combustion and conversion of the nitrogen.

TABLE 4. Random sequence of starch samples at trace level.

Run	Starch ID	Weight (mg)	N %	Protein %
1	A	1000.4	0.0177	0.1107
2	B	1002.5	0.0404	0.2528
3	C	1051.5	0.0162	0.1015
4	D	1050.4	0.0380	0.2378
5	B	1099.6	0.0404	0.2525
6	A	1100.9	0.0179	0.1120
7	E	1000.9	0.0356	0.2228
8	E	1003.9	0.0354	0.2212
9	D	1050.7	0.0382	0.2387
10	G	1050.8	0.0191	0.1196
11	G	1100.9	0.0188	0.1174
12	E	1100.3	0.0365	0.2280
13	F	1101.2	0.0143	0.0914
14	F	999.9	0.0150	0.0935
15	C	1050.9	0.0167	0.1046
16	C	1100.1	0.0162	0.1014
17	B	1102.1	0.0403	0.2519
18	D	1051.8	0.0387	0.2419
19	A	1051.0	0.0178	0.1115

TABLE 5. Statistical data of starch samples at trace level.

Starch ID	N %	RSD %	Protein %	RSD %
A	0.0177 0.0179 0.0178	0.5618	0.1107 0.1120 0.1115	0.5886
B	0.0404 0.0404 0.0403	0.1430	0.2528 0.2525 0.2519	0.1816
C	0.0162 0.0167 0.0162	1.7638	0.1015 0.1046 0.1014	1.7750
D	0.0380 0.0382 0.0387	0.9414	0.2378 0.2387 0.2419	0.8998
E	0.0356 0.0354 0.0365	1.6352	0.2228 0.2212 0.2280	1.5872
F	0.0143 0.0150	3.3787	0.0914 0.0935	3.3285
G	0.0191 0.0188	1.1194	0.1196 0.1174	1.3128

Table 6 shows the N/Protein data obtained of a slurry starch sample analysis. The liquid sample was adsorbed by the inert material Chromosorb (WAW 30/60 mesh) into the tin capsule and the sample weight used for analysis was 1.0 – 1.5 grams.

TABLE 6. Nitrogen / Protein data of slurry starch.

N %	RSD %	Protein %	RSD %
0.0355	3.1947	0.2216	3.1968
0.0352		0.2200	
0.0366		0.2291	
0.0380		0.2373	
0.0350		0.2186	
0.0354		0.2214	

An overlay of chromatograms is shown in Figure 3 to demonstrate the performance of the FLASH 4000 analyzing samples at about 100 ppm nitrogen at 1 gram. The *black chromatogram* is obtained from sample F (about 150 ppm N) with a Nitrogen Area of 173015 uV/sec while the *red chromatogram* is the blank analysis obtained with glucose at 1 gram giving a Nitrogen Area of 34223 uV/sec.

FIGURE 3. Overlay of chromatograms

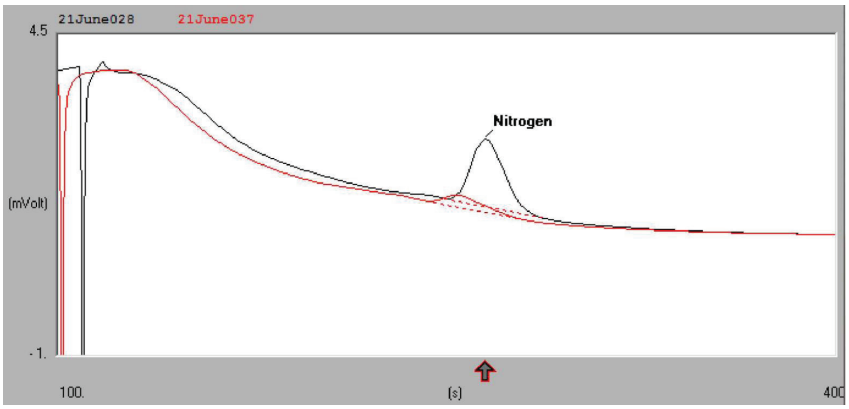


TABLE 7. Nitrogen / Protein data comparison

Sample	FLASH 4000		Kjeldahl Method	
	N %	Protein %	N %	Protein %
1	0.2527	1.5794	0.2504	1.5650
2	0.0404	0.2525	0.0399	0.2494
3	0.0358	0.2237	0.0360	0.2250

Conclusion

The FLASH 4000 analyzer demonstrates the best solution for Nitrogen / Protein determination due to:.

- Excellent reproducibility and accuracy.
- No memory effect when changing the sample and content of nitrogen.
- Nitrogen determination in a wide range from trace to high content without matrix effect.
- Combustion method is approved by official organizations (AOAC, AACC,AOCS, ISO, etc).

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