

# Analytical Comparison of the Thermo Scientific FLASH 2000 Nitrogen/Protein Analyzer with the traditional Kjeldahl Method

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## Key Words

AOAC 990.03, combustion, Kjeldahl, modified Dumas method, nitrogen, protein

## Introduction

One of the most important nutrients of food and animal feed is protein. The quantity of protein required by animals is very precise as excess amounts lead to amino acid deficiency and generate unnecessary amounts of energy. As a consequence, the exact determination of the amount of protein in animal feed is fundamental in achieving high nutritional quality of animal feed and securing the safety of final food products intended for human consumption.

In the case of fish meal, for example, the analysis of nitrogen is critical for daily quality control of production and for specification in contracts. All fish meal is traded on its protein content whether through pricing on a unit-of-protein basis or by guarantee of a minimum quantity of protein content. If the amount of nitrogen is multiplied by a factor depending on the kinds of protein expected to be present in the food, then the total protein content can be determined.

Determination of nitrogen is also very important in dietary fibre analysis as part of the enzymatic-gravimetric method to correct the fibre residue values for protein. The precise and accurate determination of nitrogen is fundamental to achieve the nutritional quality of foods, such as ready-to-eat meals, processed foods, grain and cereal products, fruits and vegetables, chocolate, fish, meat and meat products, etc. Dietary fibre and proteins are both important values in nutritional labeling and are used for the calculation of total carbohydrates.

The globalization of the food market requires accurate and reliable control of the characteristic of products for the protection of commercial value, but mainly to safeguard consumer health and manufacturer reputation. Official



Figure 1. FLASH 2000 Nitrogen/Protein Analyzer

regulations establish the protein content and labeling requirements which enable consumers to make price and quality comparisons based on % protein declarations.

For this reason the use of an accurate instrumental analytical technique is required. As the demand for improved sample throughput, reduction of operational costs and minimization of human errors is becoming more notable, it is essential to have a simple and automatic technique which allows fast analysis with excellent reproducibility, and that can avoid the risk of handling toxic chemicals. An alternative to the classical Kjeldahl method, based on Dumas (combustion) method, has been developed and approved by industry associations (AOAC, AACC, AOCS, ASBC, ISO and IFFO).

The Thermo Scientific™ FLASH 2000 elemental analyzer (Figure 1), based on the dynamic combustion of the material, requires no sample digestion or toxic chemicals, while providing important advantages in terms of time, automation and quantitative determination of nitrogen in a large range of concentration. This document presents the FLASH 2000 OEA as a powerful analytical alternative to the classical Kjeldahl method.

## Analytical Configuration

The sample is weighed in a tin capsule and introduced into the combustion reactor via the Thermo Scientific™ MAS™ 200R autosampler together with a proper amount of oxygen by OxyTune function, insuring complete combustion of the sample.

After combustion, the produced gases are carried by a helium flow to a second reactor filled with copper, then swept through CO<sub>2</sub> and H<sub>2</sub>O traps, a GC column and finally detected by a thermal conductivity detector (TCD) (Figure 2).

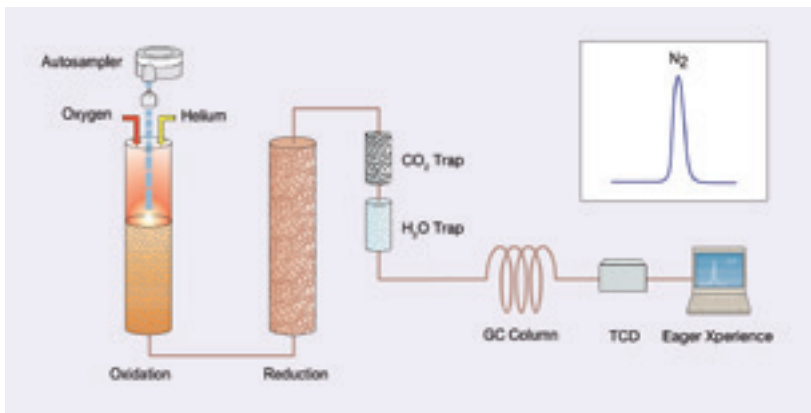


Figure 2. Nitrogen/Protein Layout

### Analytical conditions:

Left Furnace Temperature:	950°C
Right Furnace Temperature:	840°C
Oven Temperature:	50°C
Carrier Flow:	140 ml/min
Reference Flow:	100 ml/min
Standard:	EDTA (9.59 %N) or Aspartic acid (10.52 %N)
EDTA:	EthyleneDiamineTetraAcetic acid

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

## FLASH 2000 OEA performance evaluation

The performance, accuracy and precision of the FLASH 2000 analyzer was evaluated through:

1) The analysis of nicotinic acid, lysine chloride and a mixture of corn grain and soybean according to the AOAC 990.03 Performance Requirements (Association of Official Analytical Chemists) in which is indicated that the system must meet or exceed following minimum performance specification:

- System must be capable of measuring nitrogen in feed materials containing 0.2 – 20 % nitrogen.
- Accuracy of system is demonstrated by making 10 successive determinations of nitrogen in nicotinic acid and lysine chloride. Means of determinations must be within  $\pm 0.15$  of the respective theoretical values, with standard deviation  $\leq 0.15$ .
- Suitable fineness of grind is that which gives relative standard deviation (RSD)  $\leq 2.0$  % for 10 successive determinations of nitrogen in mixture of corn grain and soybean (2+1) that has been ground for analysis.  $RSD \% = (SD / \text{mean } \%N) \times 100$ . Fineness (ca. 0.5 mm) required to achieve this precision must be used for all mixed feeds and other nonhomogeneous materials.

2) The analysis of BIPEA (Bureau InterProfessionnel d'Etudes Analytiques, France) Reference Materials. The results obtained were compared with the average and range indicated in the relative Reference Materials Certificates.

3) The participation in International Round Robin Tests WEPAL (Wageningen Evaluating Programs for Analytical Laboratories, Wageningen University, Netherlands) for plants samples analysis. The data obtained with the FLASH 2000 OEA was compared with the range accepted by WEPAL statistic studies, including both Kjeldahl and nitrogen methods which include the combustion method.

## Results

Table 1 shows the reproducibility of 10 consecutive analysis of nicotinic acid, lysine chloride and a mixture of corn grain and soybean according to AOAC 990.03 Performance Requirements. The weight of sample was 50 – 100 mg for nicotinic acid and lysine chloride while for the mix of corn grain and soybean was 200-300 mg. The protein factor used to calculate the protein content was the default value 6.25 present in the Eager Xperience software.

Table 1. Reproducibility of Nitrogen / Protein determination

Sample	Nicotinic acid	Lysine chloride	Mixture of corn grain and soybean	
%	Nitrogen %	Nitrogen %	Nitrogen %	Protein %
Data	11.30	15.31	3.27	20.44
	11.30	15.23	3.25	20.29
	11.35	15.16	3.26	20.38
	11.29	15.22	3.24	20.28
	11.37	15.27	3.28	20.52
	11.42	15.11	3.27	20.42
	11.42	15.22	3.25	20.36
	11.44	15.19	3.26	20.39
	11.48	15.19	3.26	20.37
	11.39	15.25	3.28	20.49
<b>Average %</b>	<b>11.38</b>	<b>15.21</b>	<b>3.26</b>	<b>20.39</b>
<b>RSD %</b>	<b>0.576</b>	<b>0.372</b>	<b>0.404</b>	<b>0.379</b>

Table 2 and 3 show the sample information and the Nitrogen / Protein data of Bipea Reference Materials analyzed in duplicate using a sample weight of about 200-300 mg. The materials were characterized through a laboratory intercomparison using Kjeldahl and combustion methods. The protein factor used to calculate the protein content was the default value 6.25 present in the Eager Xperience software.

Table 2. BIPEA sample information available

Sample	Moisture	Fat	Carbohydrate	Kjeldahl Protein		Combustion Protein	
	%	%	%	Av.%	Tolerance	Av.%	Tolerance
Bipea - Feed for Sow	9.8	2.8	48.7	16.0	0.6	16.2	0.6
Bipea - Dehydrated Alfalfa	7.7		29.3	14.8	0.6	15.1	0.6
Bipea - Hyperproteic Powder		0.8		85.4	3.4	86.4	3.5

Table 3. Reproducibility of Nitrogen / Protein determination in BIPEA Reference Materials

Sample	Bipea - Feed for Sow		Bipea - Dehydrated Alfalfa		Bipea - Hyperproteic Powder	
	N %	Protein %	N %	Protein %	N %	Protein %
	2.60	16.25	2.45	15.31	13.65	85.31
	2.58	16.12	2.44	15.25	13.63	85.19
<b>Average %</b>	<b>2.59</b>	<b>16.185</b>	<b>2.445</b>	<b>15.28</b>	<b>13.64</b>	<b>85.25</b>
<b>RSD %</b>	<b>0.546</b>	<b>0.568</b>	<b>0.289</b>	<b>0.278</b>	<b>0.104</b>	<b>0.099</b>

Table 4 shows a comparison between the data obtained with the FLASH 2000 OEA and the range accepted by WEPAL statistic studies regarding Kjeldahl (N-Kj) and Total Nitrogen (N-To) techniques. The large number of plants shown were selected to cover a wide range of concentrations and sample matrix to verify the ability to use the combustion method instead of the classical Kjeldahl method for this application field. The weight of sample using the FLASH 2000 OEA was 200 – 300 mg.

Table 4. Nitrogen data comparison

Sample Name	WEPAL accepted range		FLASH 2000 OEA
	N – Kj %	N – To %	Nitrogen % data
Carrots (Root)	0.920 – 1.224	0.983 – 1.200	1.019
Sprouts	3.080 – 3.839	3.368 – 3.805	3.409
Broad beans (Pod)	2.261 – 2.738	2.418 – 2.769	2.466
Mango Tree (Leaves)	1.305 – 1.468	1.310 – 1.527	1.311
Summer Wheat	1.977 – 2.400	2.118 – 2.468	2.123
Parsley	3.640 – 4.172	3.668 – 4.417	3.956
Chervil	3.790 – 4.612	4.107 – 4.927	4.368
Clover (Honey-Stalk)	1.209 – 1.410	1.259 – 1.499	1.277
Chive (S)	3.392 – 3.990	3.464 – 4.211	3.739
Rose (Plant)	1.782 – 2.085	1.851 – 2.189	1.913
Rose (Plant)	1.579 – 1.820	1.627 – 1.859	1.700
Aubergine (Plant)	1.270 – 1.599	1.452 – 1.789	1.556
Grass (GR 94)	2.671 – 3.160	2.828 – 3.186	2.864
Medlar (Fruit)	0.378 – 0.503	0.407 – 0.537	0.465
Rose (Plant)	1.400 – 1.614	1.442 – 1.679	1.494
Rose (Plant)	1.180 – 1.396	1.191 – 1.491	1.283
Sprouts	1.789 – 2.029	1.849 – 2.063	1.884
Rose (Plant)	1.177 – 1.380	1.221 – 1.448	1.349
Oil Palm (Leaf)	2.398 – 2.719	2.467 – 2.849	2.571
Wheat (Straw)	0.382 – 0.519	0.260 – 0.671	0.448
Oil Palm (Leaf)	2.468 – 2.836	2.570 – 2.897	2.655
Maize (Plant)	1.589 – 1.931	1.653 – 2.050	1.843
Grass	2.289 – 2.737	2.436 – 2.750	2.482

Table 5 shows the reproducibility of 10 consecutive analysis of a Milk Reference Material from Ceca Lait (Centre d'Étude et de Contrôle des Analyses en Industrie Laitière, France). The sample weight was 100 – 200 mg and the milk was adsorbed on the inert material Chromosorb. The data obtained with the FLASH 2000 OEA are comparable with the mean certified value of 0.5284 %N obtained by 5 laboratories using Kjeldahl method. The protein factor used to calculate the protein content was 6.38.

N %	Average N %	RSD %	Protein %	Average Protein %	RSD %
0.5312	0.5298	0.5604	3.3891	3.3800	0.5605
0.5286			3.3722		
0.5321			3.3948		
0.5339			3.4065		
0.5306			3.3854		
0.5251			3.3504		
0.5335			3.4035		
0.5264			3.3584		
0.5288			3.3735		
0.5276			3.3659		

Table 6 shows a comparison of the protein data obtained of fish meal analysis with the traditional Kjeldahl method and the FLASH 2000 OEA. The small differences between the data demonstrate an optimal correlation of the techniques. The weight of sample used for the FLASH 2000 OEA was 200 – 300 mg. The protein factor used to calculate the protein content was the default value 6.25 present in the Eager Xperience software.

Table 6. Protein data comparison of fish meal samples

Fish meal sample	FLASH 2000 OEA - Protein %	Kjeldahl Method - Protein %	Difference
1	63.7	63.5	0.2
2	65.4	65.4	0.0
3	65.5	65.2	0.3
4	69.7	70.2	-0.5
5	69.8	70.0	-0.2
6	71.6	72.0	-0.4
7	69.7	69.5	0.2
8	67.9	68.5	-0.6
9	69.6	69.4	0.2
10	70.4	70.0	0.4
11	69.9	69.6	0.3
12	67.5	67.3	0.2
13	67.8	67.5	0.3
14	65.3	64.8	0.5
15	69.7	69.7	0.0
16	65.4	65.3	0.1
17	70.5	70.0	0.5
18	70.7	70.2	0.5
19	71.9	71.9	0.0
20	69.1	69.5	-0.4
21	69.9	70.0	-0.1
22	65.4	65.6	-0.2
23	67.3	67.6	-0.2
24	65.2	64.8	0.4

In the brewing industry Nitrogen / Protein determination is requested in malt, barley, beer and wort samples. Table 7 shows a comparison of nitrogen and protein data of malt and barley obtained with the traditional Kjeldahl method and the FLASH 2000 OEA while Table 8 shows a comparison of nitrogen data of beer and wort obtained with both methods. The FLASH 2000 OEA results are the average of three runs. The sample weight of malt and barley was 200 – 300 mg while the liquid samples, beer and wort, were analyzed by direct liquid injection of 125 ul of sample using the Thermo Scientific™ AS/AI 1310 liquid autosampler. The protein factor used to calculate the protein content was the default value 6.25 present in the Eager Xperience software.



Table 7. Nitrogen / Protein data comparison of malt and barley samples

Sample Name	Kjeldahl Method		FLASH 2000 OEA		
	N %	Protein %	N %	Protein %	RSD %
Malt 1	1.66	10.38	1.67	10.44	0.25
Malt 2	1.75	10.94	1.78	11.12	0.67
Malt 3	1.54	9.62	1.53	9.56	0.51
Malt 4	1.43	8.94	1.40	8.75	0.66
Barley 1	1.39	8.69	1.42	8.88	0.46
Barley 2	1.35	8.44	1.34	8.38	0.87
Barley 3	1.56	9.75	1.57	9.81	0.56
Barley 4	1.47	9.19	1.45	9.06	1.01

Table 8. Nitrogen data comparison of beer and wort samples

Sample Name	Kjeldahl Method	FLASH 2000 OEA	
	N %	N %	RSD %
Beer 1	0.0587 – 0.0592	0.0594	1.133
Beer 2	0.0641 – 0.0644	0.0647	1.023
Beer 3	0.0650 – 0.0666	0.0659	0.956
Beer 4	0.0614 – 0.0619	0.0618	1.011
Beer 5	0.0628 – 0.0630	0.0630	0.892
Beer 6	0.0640 – 0.0645	0.0637	0.912
Wort 1	0.0885 – 0.0890	0.0892	1.232
Wort 2	0.1140 – 0.1150	0.1170	0.874
Wort 3	0.1300 – 0.1310	0.1320	0.912
Wort 4	0.0995 – 0.0993	0.0993	1.112
Wort 5	0.0825 – 0.0827	0.0821	1.098
Wort 6	0.0889 – 0.0893	0.0899	1.210

Table 9 shows a Nitrogen / Protein comparison between FLASH 2000 OEA and Kjeldahl methods of different food and related products. The samples were chosen due to the different content of nitrogen, fat, carbohydrate and moisture to demonstrate excellent correlation. The analyses were performed in duplicate by the FLASH 2000 OEA.

Table 9. Comparison FLASH 2000 OEA vs Kjeldahl values of food samples

Sample	FLASH 2000 OEA		Kjeldahl Method	
	N %	Protein %	N %	Protein %
Soya	6.27	39.20	6.27	39.18
Lentils	4.35	27.17	4.35	27.19
Rice	1.13	7.08	1.12	7.00
Wheat	1.75	10.91	1.74	10.89
Beans	3.74	23.35	3.74	23.38
UHT milk 1	0.53	3.38	0.53	3.37
UHT milk 2	0.50	3.19	0.49	3.17
Crude milk 1	0.57	3.65	0.57	3.66
Crude milk 2	0.47	3.03	0.47	3.02
Crude milk 3	0.41	2.65	0.42	2.66
Pasteurized milk 1	0.50	3.21	0.50	3.19
Pasteurized milk 2	0.46	2.96	0.47	2.99
Milk powder 1	4.32	27.56	4.30	27.43
Milk powder 2	4.18	26.64	4.19	26.73
Milk powder 3	5.46	34.83	5.43	34.64
Yoghurt	0.080	0.51	0.078	0.50
Mascarpone cheese	0.635	4.05	0.638	4.07
Grapes	0.52	3.25	0.51	3.19
Bacon (low fat)	2.73	17.06	2.70	16.86
Meat loaf	2.01	12.57	1.97	12.31
Ham	2.56	16.00	2.54	15.87
Biscuits 1	1.40	8.80	1.39	8.72
Biscuits 2	1.36	8.51	1.34	8.37
Flour	1.34	8.40	1.32	8.24

## Conclusion

The results demonstrate that the Thermo Scientific FLASH 2000 analyzer is the best solution for nitrogen determination offering excellent reproducibility, with no memory effect observed when changing sample matrix.

The data obtained through the use of the instrument were within the tolerance in the Reference Materials certificates and in the range accepted for Kjeldahl and Combustion methods included in the WEPAL Round Robin Tests, indicating the high performance of the system. Providing an ideal solution for nitrogen determination, the system

offers consistent results comparable to the data obtained to the Kjeldahl method, while also removing the requirements for sample digestion or toxic chemicals. As a complete automatic system based on the combustion of the sample, the FLASH 2000 analyzer is approved by multiple official organizations and is able to accurately analyze nitrogen in a wide range from low to high content without matrix effect.

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