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High protein content determination in food and animal feed by combustion using the Thermo Scientific Flash*Smart* Elemental Analyzer

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Keywords

Animal Feed, Combustion, Food Quality, Labeling, Nitrogen, Protein

Goal

Demonstrate the Elemental Analysis as a powerful analytical alternative to the classical Kjeldahl method for the determination of high protein content in food and animal feed for food quality and labeling purposes.

Introduction

Protein is one of the most important nutrients of food and animal feed. The exact determination of the amount of protein through the determination of the nitrogen content is fundamental for the nutritional quality of animal feed and for the safety of final food products intended for human consumption.

For fish meal, for example, the analysis of nitrogen is critical for daily quality control of production and for specification. Fish meal is traded according to its high protein content whether through pricing on a unit-of-protein basis or by guarantee of a minimum protein content quantity. 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. Same issue is for soy and gluten, other two matrices with high content of protein.

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The globalization of the food market requires accurate and reliable control of products characteristic for the protection of commercial value, but mainly to safeguard consumer health and manufacturer reputation. Official regulations establish the protein content and labeling requirements, which enable consumers to define price and quality comparisons based on % protein declarations. For this reason, the use of a simple and automated technique allowing fast analysis with excellent reproducibility, and that can avoid the risk of handling toxic chemicals is required. 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, IFFO, IDF and others).

The Thermo Scientific™ Flash*Smart*™ Elemental Analyzer (Figure 1), based on the dynamic combustion of the material (Dumas method), 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.

Figure 1. Flash*Smart* N/Protein Elemental Analyzer.

Method

The Elemental Analyzer operates according to the dynamic flash combustion of the sample. Samples are weighed in tin containers and introduced into the combustion reactor via the MAS Plus Autosampler with oxygen. After combustion, the produced gases are carried by a helium flow to a second reactor filled with copper, then swept through CO_2 and H_2O traps, a GC column and finally detected by a Thermal Conductivity Detector (TCD) (Figure 2). A comprehensive report is automatically generated by the Thermo Scientific™ Eager*Smart*™ Data Handling Software. From the nitrogen data obtained and a protein factor, the dedicated software allows the automated calculation of the protein content.

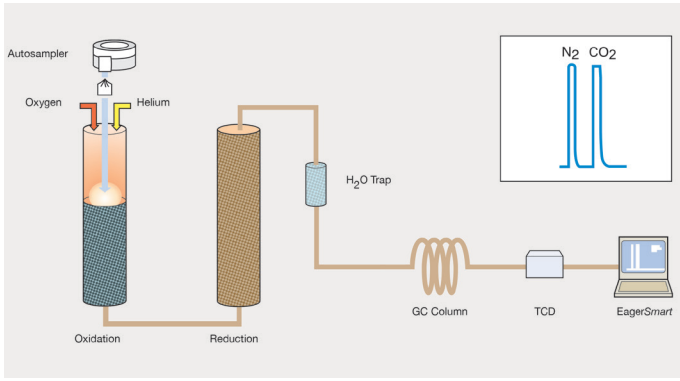

Figure 2. Nitrogen configuration.

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	Nicotinamide (22.94 N %)

Note: The oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune function in the Eager*Smart* Data Handling Software.

Results

Samples were selected representing a range of varying nature in order to evaluate the performance of the system for higher Protein (40–90 protein %). The data obtained demonstrates the no-matrix effect in the determination of nitrogen, indicating complete combustion for all types of samples. For most of the samples, the protein factor used to calculate the protein content was 6.25 while for milk protein concentrates the protein factor was 6.38.

The accuracy and precision of the Flash*Smart* EA was evaluated through the analysis of a BIPEA (Bureau InterProfessionnel d'Etudes Analytiques, France) Reference Material. The results obtained were compared with the average and range indicated in the relative Certificate.

The materials were characterized through a laboratory intercomparison using Kjeldahl and combustion methods. Table 1 shows the sample information included in the certificate and the data obtained with Flash*Smart* EA analyzing the sample in duplicate with a sample weight of about 200–300 mg.

Table 1. BIPEA hyperproteic powder.

BIPEA hyperproteic powder					FlashSmart EA		
Fat	Protein Values		Protein Values		N%	Protein %	RSD%
%	Av. %	Tolerance	Av. %	Tolerance			
0.8	85.4	3.4	86.4	3.5	13.65 13.63	85.31 85.19	0.10

Table 2 shows the N/Protein repeatability of a soy sample while Table 3 shows the N/Protein data of soy products. Sample weight was 200–300 mg for solid samples while for the cream colored soy protein isolate the weight was 150–160 mg.

The dehulled soy bean meal is for animal consumption while the soy protein concentrate is for human food industries. The cream colored soy protein isolate is applicable for a wide range of food products requiring particularly high protein and bland flavor profile, providing gel strength and emulsifying properties.

Table 2. N/Protein repeatability of soy sample.

Run	W (mg)	N%	RSD%	Protein %	RSD%
1	205.9	7.96		49.74	
2	296.6	7.92		49.74	
3	217.4	7.95		49.67	
4	200.2	7.85		49.06	
5	232.4	7.88		49.28	
6	230.2	7.95		49.70	
7	211.8	7.88		49.26	
8	216.0	7.94	0.66	49.61	0.66
9	242.5	7.84		49.00	
10	202.9	7.89		49.33	
11	218.0	7.99		49.97	
12	198.7	7.97		49.82	
13	204.0	7.86		49.11	
14	220.1	7.98		49.85	
15	206.2	7.85		49.05	

Table 3. N/Protein data of soy products.

Sample	N%	RSD%	Protein %	RSD%
Soy	7.89 7.87 7.90	0.19	49.29 49.20 49.39	0.19
Soybean meal 1	7.88 7.87 7.88	0.07	49.22 49.21 49.24	0.03
Soybean meal 2	7.71 7.70 7.72	0.13	48.20 48.10 48.25	0.16
Soybean meal 3	7.71 7.70 7.72	0.08	46.60 46.71 46.69	0.13
Soy protein meal	10.65 10.65 10.63	0.11	66.54 66.57 66.42	0.12
Soy protein powder	8.24 8.26 8.25	0.17	51.48 51.65 51.57	0.17
Dehulled soybean meal	7.68 7.65 7.69	0.27	48.00 47.81 48.06	0.27
Soy protein concentrate	10.53 10.50 10.51	0.15	65.81 65.63 65.72	0.14
Cream colored soy protein isolate	13.95 13.95 13.94	0.04	87.20 87.19 87.13	0.04

Table 4 shows the N/Protein repeatability of gluten samples while Table 5 shows the N/Protein data obtained of several animal feed samples. The samples were weighted at 200–300 mg.

Table 4. N/Protein data of gluten samples.

Sample	N%	RSD%	Protein %	RSD%
Gluten 1	10.97 10.96 10.91	0.29	68.54 68.51 68.16	0.31
Gluten 2	9.83 9.81 9.85	0.20	61.44 61.33 61.56	0.19
Corn gluten 1	10.05 10.04 10.05	0.03	62.81 62.79 62.82	0.03
Corn gluten 2	9.47 9.48 9.47	0.02	59.21 59.23 59.22	0.02
Corn gluten 3	10.42 10.43 10.44	0.09	65.13 65.22 65.24	0.09
Corn gluten 4	10.75 10.77 10.77	0.12	67.19 67.33 67.33	0.12
Corn gluten 5	10.38 10.38 10.37	0.02	64.88 64.87 64.86	0.02
Gluten meal 1	11.29 11.25 11.27	0.18	70.57 70.32 70.47	0.18
Gluten meal 2	9.81 9.81 9.81	0.00	61.30 61.31 61.30	0.01

Table 5. N/Protein data of gluten animal feed samples.

Sample	N%	RSD%	Protein %	RSD%
Poultry meal 1	8.41 8.43 8.42	0.12	52.59 52.66 52.62	0.07
Poultry meal 2	10.06 10.04 10.01	0.25	62.90 62.73 62.57	0.26
Feather meal	12.69 12.71 12.71	0.09	79.34 79.44 79.43	0.07
Pet food	13.98 13.93 13.93	0.21	87.38 87.07 87.06	0.21
Horse animal feed 1	10.37 10.38 10.32	0.31	64.82 64.86 64.52	0.29
Horse animal feed 2	7.42 7.51 7.48	0.61	46.39 46.95 46.78	0.61

Table 6 shows the N/Protein repeatability of meat related samples while Table 7 shows the N/Protein data obtained of different fish and krill meal samples analyzed in triplicate. The sample was weighted at 100–250 mg. Table 8 shows a comparison of the protein data obtained of fish meal analysis with the traditional Kjeldahl method and the Flash*Smart* EA. The small differences between the data demonstrate an optimal correlation of the techniques.

Table 6. N/Protein data of meat related samples.

Sample	N%	RSD%	Protein %	RSD%
Meat protein powder	13.65 13.65 13.64	0.04	85.32 85.30 85.26	0.04
Meat meal 1	8.91 8.94 8.98 8.93 8.99 8.97 8.92 8.98 8.92 8.90 8.89 8.89	0.41	55.69 55.84 56.12 55.80 56.20 56.07 55.77 56.11 55.76 55.59 55.56 55.55	0.42
Meat meal 2	7.83 7.88 8.00 7.90 7.79 7.92 7.83 7.95 8.03 7.89 7.96 7.91	0.88	48.96 49.24 50.00 49.40 48.70 49.53 48.96 49.68 50.19 49.29 49.73 49.41	0.88
Mix of bone, meat and fat	9.76 9.77 9.60 9.46 9.60 9.98 9.74 9.73 9.72 9.64	1.42	60.99 61.08 60.02 59.11 60.02 62.36 60.86 60.80 60.75 60.23	1.42

Table 7. N/Protein data of fish and krill meal samples.

Sample		N%	Protein %	RSD%
Fish meal	1	10.19	63.69	0.33
	2	10.47	65.45	0.90
	3	11.14	69.56	0.33
	4	11.51	71.91	0.57
	5	11.29	70.55	0.09
Krill meal	1	9.95	62.21	0.68
	2	9.12	57.01	1.05
	3	9.90	61.87	0.38
	4	9.27	57.94	0.51
	5	9.26	57.87	1.45

Table 8. Protein comparison data of fish samples between Flash*Smart* EA and Kjeldahl methods.

Fish meal sample	Flash*Smart* EA protein %	Kjeldahl 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	64.4	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

Different types of milk protein concentrates were analyzed to evaluate the repeatability. The calibration was performed with 80–100 mg of aspartic acid. The protein content was calculated using the protein factor of 6.38. Table 9 shows the protein results obtained with Milk Protein Concentrate samples weighing 80–100 mg analyzed in duplicate.

Table 9. N/Protein data of milk protein concentrate samples.

Sample	Weight (mg)	N%	Protein %	RSD%
1	82.7 83.3	12.199 12.246	77.83 78.13	0.42
2	84.7 85.6	12.324 12.237	78.63 78.07	0.79
3	83.4 84.3	12.970 12.958	82.75 82.67	0.11
4	83.1 83.8	12.824 12.815	81.82 81.76	0.08
5	85.8 85.6	12.929 12.911	82.49 82.37	0.17
6	84.6 82.37	12.373 12.378	78.94 78.97	0.04
7	83.1 83.8	12.260 12.299	78.22 78.47	0.35
8	87.5 88.6	12.426 12.448	79.28 79.42	0.20
9	84.3 84.6	12.933 12.987	82.51 82.86	0.49
10	83.6 83.7	12.519 12.536	79.87 79.98	0.16
11	86.4 86.8	12.418 12.414	79.23 79.20	0.04

At last, three egg albumin samples were analyzed to evaluate the repeatability of the data weighing 150–200 mg of sample. Table 10 shows the N/Protein data obtained.

Table 10. N/Protein data of egg albumin samples.

Sample	N%	RSD%	Protein %	RSD%
1	13.17 13.18 13.17	0.04	82.34 82.35 82.30	0.03
2	13.09 13.08 13.08	0.04	81.84 81.72 81.75	0.08
3	13.26 13.23 13.27	0.16	82.85 82.70 82.96	0.16

Conclusions

The Thermo Scientific Flash*Smart* EA, based on the combustion method, demonstrates to be an excellent solution for nitrogen/protein determination of samples with high protein content due to the superior repeatability obtained, with no memory effect observed when changing the type of sample. This indicates the complete and accurate detection of the nitrogen content.

The Dumas Combustion method has been approved and adopted by Official Organizations as ASBC, AOAC, AACC, AOCS, ISO, IFFO and IDF.

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