APPLICATION NOTE

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Nitrogen/Protein Determination in Food and Animal Feed by Combustion Method (Dumas) using the Thermo Scientific Flash*Smart* Elemental Analyzer

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Key Words
Accuracy, Automation, Flash Combustion, Nitrogen, Protein, Repeatability, Unattended Analysis

Goal

To demonstrate the performance of the Thermo Scientific Flash*Smart* Elemental Analyzer for nitrogen/protein determination in food and animal feed.

Introduction

Understanding the nutritional composition of food and animal feed plays a very important role in industry for research and quality control purposes.

Figure 1. Thermo Scientific Flash*Smart* Analyzer.

The globalized market for food products requires accurate control of product characteristics in order to protect commercial value, to safeguard consumer health and manufacturer reputation. Official regulations establish protein content and labelling requirements, which enable consumers to define price and make quality comparisons. This means that protein analysis is an issue of significant economic and social interest because of the legal, nutritional, health, safety and economic implications for the food and animal feed industries. A common test used in the production process to determine the protein content is nitrogen analysis which is periodically monitored.

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Therefore, it is very important to have an accurate and precise analytical technique, ideally with full automation, which allows fast analysis with excellent reproducibility whilst avoiding the use of toxic chemicals. The technique must be robust and capable of analyzing fresh and processed products in various physical states (powders, slurries, dilute liquids, emulsions, gels, pastes) and deal effectively with products from either animal or plant sources.

An alternative to the classical Kjeldahl method, based on the Dumas (combustion) method, has been developed. The Dumas assay precedes Kjeldahl analysis by more than 50 years. The former technique was invented by Jean Baptiste Dumas. In recent decades, the advent of easy-to-use and highly accurate combustion nitrogen analyzers rekindled interest in the Dumas method. The Dumas combustion method is comparatively quicker, cheaper, easier to perform, safer and more environment friendly. The Dumas method is approved by different associations (AOAC, AACC, AOCS, ASBC, ISO and IFFO).

The Thermo Scientific™ Flash*Smart*™ Analyzer (Figure 1), based on the dynamic combustion method (modified Dumas method), provides rapid and automatic nitrogen determination with high accuracy and precision comparable with traditional methods, without use of hazardous chemicals.

The Flash*Smart* Analyzer allows you to analyze high and low nitrogen amounts without matrix effect. Sample protein content is calculated automatically using a conversion factor in the Thermo Scientific™ Eager*Smart*™ Data Handling Software.

Methods

The Flash*Smart* Analyzer operates according to the dynamic flash combustion of the sample. Powdered samples were weighed in tin containers while liquid samples were weighed in tin containers adsorbed on the inert material Chromosorb®, both were introduced into the combustion reactor from the Thermo Scientific™ MAS Plus Autosampler together with oxygen determined by the Thermo Scientific™ OxyTune™ Function. The Thermo Scientific OxyTune Function automatically evaluates the oxygen required for combustion, according to the weight and nature of the sample. After combustion, the produced gases are conveyed 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 complete report is automatically generated by the dedicated Eager*Smart* Data Handling Software and displayed at the end of the analysis. From the nitrogen data obtained combined with a protein factor, the software allows the automatic calculation of the protein content.

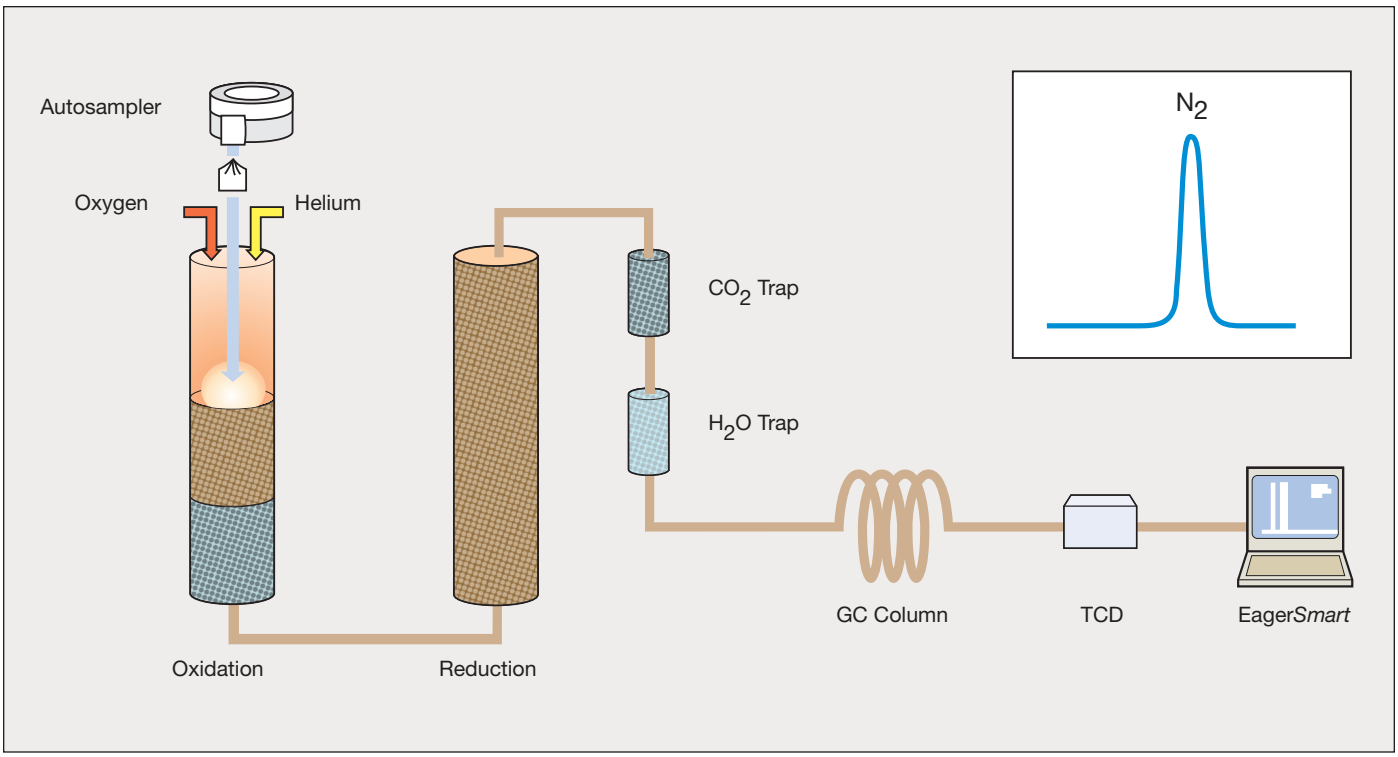

Figure 2. Nitrogen configuration of the Flash*Smart* EA.

Results

The accuracy and precision of the Flash*Smart* Analyzer was evaluated by analyzing the Thermo Scientific Pasta Reference Material on five different instruments and by the analysis of BIPEA (Bureau InterProfessionnel d'Etudes Analytiques, France) Reference Materials. The results obtained were compared with the average and range values indicated in the Reference Materials Certificates.

Five instruments were chosen to perform the analytical test of nitrogen determination in Pasta Reference Material. The calibration was performed with 50-100 mg Aspartic acid standard using K factor as the calibration method. Following, three analyses of 200-300 mg Pasta Reference Material were performed as unknown. Table 1 shows the correlation between the certified Nitrogen percentages, the uncertainty and the experimental data obtained in triplicate for each instrument. All data fall within the certified range and they are comparable confirming the repeatability and reproducibility expected.

Table 1. Nitrogen data of Pasta Reference Material.

Technical Specification			Flash*Smart* Analyzers									
Element	Theoretical %	Uncertainty (±)	1		2		3		4		5	
			%	RSD %	%	RSD %	%	RSD %	%	RSD %	%	RSD %
N	2.20	0.07	2.18 2.17 2.17	0.26	2.19 2.18 2.18	0.26	2.20 2.19 2.19	0.26	2.18 2.19 2.18	0.26	2.18 2.20 2.20	0.26

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.

Table 2. BIPEA sample information.

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

Table 3. Nitrogen/Protein data of BIPEA Reference Materials by Flash*Smart* Analyzer.

BIPEA Ref. Mat.	Feed for Sow		Dehydrated Alfalfa		Hyperproteic Powder	
%	N %	Protein %	N %	Protein%	N %	Protein %
	2.61	16.31	2.43	15.21	13.74	85.90
	2.61	16.34	2.42	15.13	13.72	85.70
Average %	2.61	16.32	2.42	15.17	13.73	85.84
RSD %	0.00	0.13	0.29	0.37	0.10	0.10

Several food and animal feed samples were selected representing a range of varying nature in order to evaluate the performance of the system in a large range of nitrogen/protein content. The data obtained demonstrates that there is no matrix effect in the determination of nitrogen, indicating complete combustion for all types of samples. For most samples, the protein factor used to calculate the protein content was 6.25 while for milk derivates samples the protein factor was 6.38.

The calibration was performed with 50-100 mg Aspartic acid and Nicotinamide standards using K factor as the calibration method. Table 3 shows the repeatability of nitrogen/protein data of food and related samples while Table 4 shows the repeatability of nitrogen/protein data of animal feed and related samples.

Table 3. Repeatability of nitrogen/protein data of food and related samples.

Sample	Weight (mg)	N %	RSD %	Protein %	RSD %
Corn	130 - 140	1.34 1.33 1.33	0.39	8.38 8.31 8.33	0.44
Oats	250 - 300	0.760 0.763 0.762	0.20	4.76 4.77 4.76	0.07
Cheese	200 - 300	4.95 4.96 4.97	0.12	30.96 31.00 31.07	0.18
Baby Milk Powder	200 - 220	1.47 1.46 1.46	0.39	9.21 9.12 9.11	0.60
Biscuit (20 % fat)	70 - 80	1.15 1.16 1.18	1.31	7.17 7.26 7.39	1.52
Vegetable Burger	200 - 300	0.656 0.655 0.654	0.15	4.10 4.09 4.09	0.16
Soy Cutlet	200 - 300	2.48 2.49 2.48	0.20	15.55 15.59 15.54	0.20
Concentrated Chicken	200 - 300	6.45 6.48 6.41	0.54	40.32 40.50 40.06	0.56
Blend Green Coffee	200 - 300	2.40 2.41 2.40	0.17	14.99 15.05 15.01	0.19
Cured Ham	300 - 350	4.38 4.41 4.39	0.35	27.37 27.56 27.44	0.35
Coppa (Meat Product)	300 - 350	4.49 4.42 4.44	0.81	28.06 27.63 27.75	0.80
Diet Crunch	180 - 230	1.80 1.82 1.81	0.55	11.24 11.37 11.31	0.57
Body Fit Crunch	200 - 250	7.70 7.70 7.69	0.07	48.10 48.14 48.05	0.09
Nuts Mix	50 - 60	4.18 4.19 4.20	0.24	26.12 26.16 26.24	0.23
Sesame Seeds	70 - 80	4.00 3.99 3.99	0.15	25.02 24.97 24.94	0.15
Tomato Soup Dried	140 - 180	1.62 1.63 1.63	0.55	10.10 10.17 10.21	0.55
Indian Chilli Sauce	200 - 250	0.056 0.057 0.055	1.79	0.352 0.354 0.345	1.35

Table 4. Repeatability of nitrogen/protein data of animal feed and related samples.

Sample	Weight (mg)	N %	RSD %	Protein %	RSD %
Layer Feed	200 - 300	2.73 2.72 2.70	0.56	17.06 17.02 16.88	0.56
Broiler Meal	200 - 300	3.67 3.68 3.68	0.16	22.91 22.99 23.03	0.27
Rapeseed	200 - 300	5.75 5.74 5.76	0.17	35.94 35.90 36.02	0.17
Corn Gluten Feed	200 - 300	3.73 3.74 3.74	0.16	23.32 23.35 23.39	0.15
Corn	200 - 300	1.23 1.23 1.23	0.00	7.69 7.69 7.71	0.15
DDGS*	200 - 300	4.53 4.55 4.54	0.22	28.30 28.46 28.38	0.28
Sheep Food	200 - 300	2.99 2.97 2.97	0.39	18.66 18.59 18.56	0.28
Sunflower Seed	200 - 300	6.18 6.16 6.18	0.19	38.60 38.48 38.63	0.21
Fibre Feed	150 - 190	3.37 3.45 3.41	1.17	21.07 21.54 21.29	1.10
Pet Food 1	200 - 300	1.08 1.07 1.08	0.86	6.77 6.68 6.78	0.86
Pet Food 2	200 - 300	5.24 5.23 5.27	0.34	32.78 32.71 32.93	0.34
Horse Food 1	200 - 300	3.59 3.64 3.59	0.80	22.43 22.76 22.41	0.87
Horse Food 2	200 - 300	1.75 1.76 1.77	0.57	10.93 11.00 11.09	0.73
Horse Food 3	200 - 300	0.55 0.54 0.55	1.06	3.44 3.35 3.42	1.39
Horse Food 4	200 - 300	3.18 3.21 3.20	0.48	19.91 20.08 20.02	0.43
Horse Food 5	200 - 300	4.78 4.79 4.76	0.32	29.87 29.94 29.77	0.29
Horse Food 6 (liquid)	180 - 200	1.42 1.41 1.40	0.71	8.87 8.83 8.73	0.73

*Dried Distillers Grains with Solubles

In addition, two tests were performed to show the accuracy and repeatability with low and high nitrogen content.

For low nitrogen determination, starch and starch slurry were selected. The calibration was performed with 50-100 mg Aspartic acid standard using K factor as the calibration method. Following, starch was weighed at 200-300 mg while for starch slurry 300-320 mg was weighed and adsorbed on Chromosorb®. Table 5 shows the data obtained.

For high nitrogen determination, different sample natures were selected. The calibration was performed with 70-100 mg Nicotinamide standard using K factor as the calibration method. Then samples were weighed at 80-300 mg and analyzed in triplicate. Table 6 shows the data obtained.

Table 5 shows. N/Protein repeatability at low nitrogen content.

Sample	N %	RSD %	Protein %	RSD %
Starch 1	0.0484 0.0486 0.0483	0.31	0.3024 0.3040 0.3016	0.40
Starch 2	0.0360 0.0367 0.0362	0.99	0.2250 0.2294 0.2262	1.00
Starch Slurry	0.0166 0.0165 0.0159	2.32	0.1038 0.1029 0.0994	2.28

Table 6 shows. N/Protein repeatability at high nitrogen content.

Sample	N %	RSD %	Protein %	RSD %
Soy	7.89 7.87 7.90	0.19	49.29 49.20 49.39	0.19
Soybean Meal	7.88 7.87 7.88	0.07	49.22 49.21 49.24	0.03
Soy Protein Concentrate	10.53 10.50 10.51	0.15	65.81 65.63 65.72	0.14
Gluten	9.83 9.81 9.85	0.20	61.44 61.33 61.56	0.19
Corn Gluten	10.05 10.04 10.05	0.03	62.81 62.79 62.82	0.03
Meat Meal	8.99 8.95 8.97	0.22	56.19 55.94 56.06	0.22
Fish Meal	11.42 11.40 11.45	0.22	71.37 71.25 71.56	0.22
Milk Protein Concentrate	12.25 12.28 12.26	0.12	76.56 75.75 76.62	0.13

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Conclusions

The Thermo Scientific Flash*Smart* Analyzer, based on the combustion method (Dumas), is an excellent solution for nitrogen/protein determination of all type of raw and final food and animal feed samples, including low to high nitrogen/protein content materials. The excellent repeatability obtained, no memory effect observed when changing the type of sample, automation and low cost per analysis combine to make the Analyzer an outstanding solution. The data presented here demonstrates the complete and accurate detection of the nitrogen in a variety of sample matrices.

The relative standard deviation was less than 2%, in accordance with the demands of AOAC methods.

The Dumas Combustion method has been approved and adopted by Official Organizations. Some relevant examples are shown in the Table 7.

Table 7. Most relevant Official Methods.

Official Organization	Method
AACC (American Association of Cereal Chemists)	Crude Protein in Cereal, 46-30, 1999.
AOAC (Association of Official Analytical Chemists)	Official Method 990.03. Protein (crude) in Animal Feed 4.2.08 Official Method 992.15. Crude Protein in Meat and Meat Products including Pet Foods 39.1.16 Official Method 992.23. Crude Protein in Cereal Grains and Oilseeds 32.2.02
AOCS (American Oil Chemists Society)	Official Method Ba 4e-93 (revised 1995). Combustion method for determination of Crude Protein
ASBC (American Society of Brewing Chemists)	Official method 1996. Nitrogen determination in Barley. Total Nitrogen in Wort and Beer by combustion method.
Office International de la Vigne et du Vin	Quantification of Total Nitrogen by Dumas Method (must and wines)
IFFO (International Fishmeal and Fish Oil organization Ltd.)	Nitrogen determination in fish meal by combustion method.
ISO 14891 (International organization for Standarization) – FIL 185 (International Dairy Federation)	Nitrogen determination in dairy products by combustion method.
ISO 16634-1, 2008 (International organization for Standarization)	Food products – Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content. Part 1: Oil seeds and animal feeding stuffs.

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