thermoscientific

Nitrogen/Protein Determination by Flash*Smart* Elemental Analyzer for Food Quality and Labeling

Liliana Krotz, Francesco Leone and Guido Giazzi, Thermo Fisher Scientific, Strada Rivoltana, 20090 Rodano (MI), Italy

ABSTRACT

Protein determination is used for the evaluation of food and beverage product quality and potentially for the uncovering of potential mislabeling cases in food and animal feed. Elemental Analysis is a powerful analytical technique performing nitrogen determination for the determination of protein content and it is an alternative to the classical Kjeldahl Method. This poster shows the performance of the Thermo Scientific Flash*Smart* Elemental Analyzer for N/Protein determination in different nitrogen concentrations in food and animal feed reference materials, showing the advantages of elemental analysis over Kjeldahl Method.

INTRODUCTION

The nutritional composition of food and animal feed is regularly analyzed by food and feed manufacturers to comply with official regulations. Regulations regarding processed food and raw foods require a series of tests aimed at determining product quality and at preventing food frauds. One of the tests is the determination of protein content of food and animal feed. The exact determination of the amount of protein, through the determination of the nitrogen content, is fundamental to define the nutritional features of animal feed and for the safety of final food products intended for human consumption. Official regulations establish the protein content and labeling requirements, which enable consumers to perform price and quality comparisons based on % protein declarations. The Kjeldahl Method is the classical technique for this analysis, even though it is recognized not to meet modern laboratories requirements. For this reason, the use of an 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 is the Dumas (combustion) method, which is comparatively quicker, cheaper, easier to perform, safer and more environmental friendly. The Dumas method is approved by different associations (AACC, AOAC, AOCS, ASBC, IDF, IFFO and ISO). The technique is robust and capable of analyzing fresh and processed products in various physical states (powders, slurries, dilute liquids, emulsions, gels, pastes and other) and deal effectively with products from either animal or plant sources.

The Thermo Scientific™ Flash*Smart* ™ Elemental Analyzer (Figure 1), based on the dynamic combustion method (modifed Dumas method), provides rapid and automated nitrogen determination without use of hazardous chemicals and offers advantages over traditional methods. The Flash*Smart* EA allows analyses of high and low nitrogen levels with no configurational change and without matrix effect. The protein content is calculated automatically using the dedicated conversion factor in the Thermo Scientific™ Eager*Smart*™ Data Handling Software.

Figure 1. Flash*Smart* Elemental Analyzer.

METHODS

The Flash*Smart* Analyzer operates according to the dynamic flash combustion of the sample. Samples were weighed in tin containers and introduced into the combustion reactor via the Thermo Scientific™ MAS Plus Autosampler with the oxygen determined by the proprietary Thermo Scientific OxyTune® feature. It allows the automated evaluation of the oxygen needed for combustion, according to the weight and nature 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_2 and H_2O traps, a GC column. Finally they are detected by a Thermal Conductivity Detector (TCD) (Figure 2). The report is automatically generated by the Thermo Scientific™ Eager*Smart* ™ Data Handling Software.

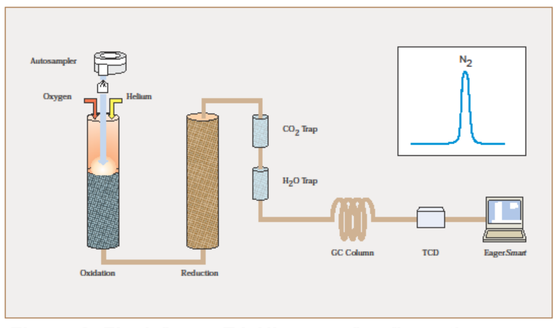

Figure 2. Flash*Smart* EA Nitrogen Configuration.

RESULTS

The accuracy and precision of the Flash*Smart* Analyzer was evaluated through the analysis of BIPEA Reference Materials (Bureau InterProfessionnel d'Etudes Analytiques, France). The results were compared with the average and range indicated in the relative certificates. The calibration was performed with 50-100 mg aspartic acid standard using K factor as calibration method.
Table 1 and 2 show the sample information and the N/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 1. BIPEA sample information.

BIPEA Ref.Mat.	Moisture %	Fat %	Carbohydrate %	Kjeldahl Protein		Combustion Protein	
				Av.	±	Av.	±
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 2. N/Protein data of BIPEA Ref. Mat. by Flash*Smart* EA.

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
Av. %	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

Food and animal feed were selected representing a range of varying nature, to evaluate the performance of the system with samples with a large range of N/Protein content. The data obtained demonstrates the no-matrix effect in the determination of nitrogen, indicating complete combustion for all samples.

The calibration was performed with 50-100 mg aspartic acid and nicotinamide standards using K factor as calibration method. For most of the samples, the protein factor used to calculate the protein content was 6.25 while for milk derivate samples the protein factor was 6.38.

Table 3 shows the repeatability of N/Protein data of food and related samples while Table 4 shows the repeatability of N/Protein data of animal feed and related samples.

Table 3. N/Protein data of food samples with Elemental Analysis.

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	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
Pasta Ref.Mat.	200-300	2.21 2.20 2.20	0.26	13.84 13.77 13.74	0.37

Table 4. N/Protein data of animal feed 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 23.34 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 Feed	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 Feed 1	200-300	3.59 3.64 3.59	0.80	22.43 22.76 22.41	0.87
Horse Feed 2	200-300	1.75 1.76 1.77	0.57	10.93 11.00 11.09	0.73
Horse Feed 3	200-300	0.55 0.54 0.55	1.06	3.44 3.35 3.42	1.39
Horse Feed 4	200-300	3.18 3.21 3.20	0.48	19.91 20.08 20.02	0.43
Horse Feed 5	200-300	4.78 4.79 4.76	0.32	29.87 29.94 29.77	0.29
Horse Feed 6	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 of the Elemental Analyzer at 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 calibration method. Then starch is weighed at 200-300 mg were analyzed while for starch slurry 300-320 mg were weighed adsorbed on Chromosorb. Table 5 shows the results obtained.

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

Table 5. 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. 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

CONCLUSIONS

For the N/Protein determination of all type of raw and final food and animal feed, the application showed that the Dumas Method meets manufacturers and laboratories requirements, including the compliance to official methods.

The Thermo Scientific Flash*Smart* Elemental Analyzer, based on the combustion method (Dumas) offers advantages over the Kjeldahl Method for the N/Protein determination in terms of automation, easy to use and cost per sample. No memory effect was observed when changing the sample. This indicates the complete and accurate detection of the nitrogen present. Also, the Flash*Smart* EA performs analysis with excellent repeatability. In all cases the relative standard deviation was less than 2%, according to the AOAC methods.

The Dumas Combustion method has been approved and adopted by Official Organizations as showed in the Table 7.

Table7. Most relevant Official Methods (partial list).

Official Organization	Method
AACC (American Association of Cereals 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 4° -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
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
Office International de la Vigne et du Vin	Quantification of Total Nitorgen by Dumas Method (must and wines)

TRADEMARKS/LICENSING

©2017 Thermo Fisher Scientific Inc. All rights reserved. AOAC is a trademark of The Association of Official Analytical Chemists; AOCS is a trademark of The American Oil Chemists' Society; AACC is a trademark of The American Association of Cereal Chemists; ASBC is a trademark of The American Society of Brewing Chemists; BIPEA is a trademark of Bureau InterProfessionnel d'Etudes Analytiques, France; IFFO is a trademark of The International Fishmeal and Fish Oil Organization. ISO is a trademark of the International Standards Organization. All trademarks are the property of Thermo Fisher Scientific.

PO42280 - Presented at the Food Integrity 2017 Conference, Parma, IT. 10-11th May 2017

ThermoFisher SCIENTIFIC