Nitrogen/Protein Determination of Insect Food and Animal Feed by Dumas Method

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ABSTRACT

Purpose: Nitrogen/Protein determination by Dumas combustion method.

Methods: Insect food and animal feed samples were analyzed through an elemental analyzer with automated autosampler.

Results: Nitrogen/Protein data are presented to assess the performance of the elemental analyzer using helium and argon as carrier gas in alternative to the Kjeldahl method.

INTRODUCTION

The nutritional properties in food and animal feed is essential for consumers. Regulations at national level require food labeling with nutritional facts, so to allow transparent quality/price comparisons. To ensure a transparent labeling, protein analysis is key from legal, nutritional, health, safety and economical points of view.

Lately, insect-based food have been legalized in some countries (for example France, Switzerland and some Asian countries) and their demand has increased. The pros of insect-based food is their high value in proteins and low value in fats and they are easy to produce. Unfortunately, the price isn't competitive and they are more expensive than other alternatives. Insect-based food success has been seen in feeding animals, fishes for the most.

The precise and accurate determination of the protein amount, through the determination of nitrogen, is key to achieve the nutritional quality of animal finished products. For these reasons, the capabilities of the Dumas method (combustion method) for the determination of nitrogen have been greatly improved to make faster, safer and more reliable than the traditional Kjeldahl method.

Combustion Dumas method has been approved and adopted by the Association of Official Analytical Chemists (AOAC Official Method 990.03. Protein crude in Animal Feed 4.2.08). In this light, the Thermo Scientific™ Flash*Smart* ™ Elemental Analyzer, based on the dynamic flash combustion of the sample, copes with a wide array of important requirements of laboratories such as accuracy, day by day reproducibility and high sample throughput.

The Flash*Smart* EA uses helium as carrier gas, which ensures high sensitivity. Considering the need for cost efficiencies and the likely increase in helium gas cost, due to its possible shortage, an alternative for the carrier gas, is needed. Argon which is readily available, can be used as alternative to helium in the Flash*Smart* EA.

Figure 1. Flash*Smart* Elemental Analyzer.

MATERIALS AND METHODS

The Flash*Smart* Elemental Analyzer operates according to the dynamic flash combustion of the sample. The sample is weighed in tin containers and introduced into the combustion reactor via the Thermo Scientific™ MAS Plus Autosampler with oxygen.

After combustion, the produced gases are carried by an helium or argon 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 Thermo Scientific™ Eager*Smart*™ Data Handling Software and displayed at the end of the analysis. The dedicated software converts automatically the nitrogen content in protein content, by using a specific protein factor.

The Eager*Smart* Data Handling Software controls all analytical parameters of the instrument including the oxygen flow and the timing of oxygen injection. It calculates automatically the amount of oxygen, relative to the sample matrix and sample weight, through the dedicated Thermo Scientific™ OxyTune™ Function ensuring the complete combustion of the sample. Through this optimization also decreases the cost per analysis by not wasting oxygen or consuming the copper unnecessarily. Figure 3 shows the OxyTune Categories.

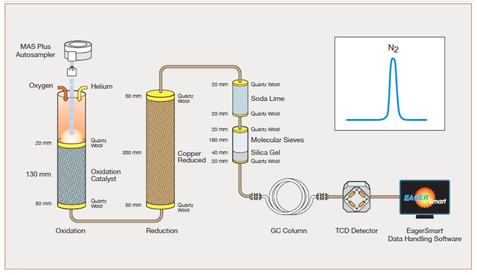


Figure 2. Nitrogen/Protein configuration.

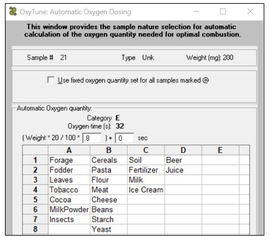


Figure 3. OxyTune Eager*Smart* Data Handling Software window.

RESULTS

Several insect-based food and animal feed samples in a large range of nitrogen concentration were analyzed to demonstrate the performance of the Flash*Smart* Elemental Analyzer using helium and argon as carrier gas. The protein factor 6.25 was used to calculate the protein content.

The instrument calibration was performed with nicotinamide standard (22.94 N%) using K factor as calibration method. The calibration was evaluated by the analysis of nicotinamide and aspartic acid as unknown before and after the samples. The data obtained fall within the technical specification of the system for nicotinamide (theoretical 22.94 N%, accepted range 22.72 – 23.16 N%), and for aspartic acid (theoretical 10.52 N%, accepted range 10.42 – 10.62 N%).

Most of the insect food and animal feed samples were homogenized by a ball mill. Table 1 shows the sample weight, the standard and the OxyTune Category when helium or argon is used as carrier gas.

Table 1. Samples, standard and OxyTune information.

Sample Name	Helium carrier gas			Argon Carrier gas		
	Sample Weight (mg)	OxyTune Category	Calibration	Sample Weight (mg)	OxyTune Category	Calibration
Microdélices Classic Nature Tenebrio	200 - 220	A	Nicotinamide 50-100 mg	120 - 130	A	Nicotinamide 50-70 mg
Microdélices Classic Nature Sigillatus	210 - 230	A		120 – 135	A	
Microdélices Apéro Tenebrio Barbecue	200 – 215	A		120 – 135	A	
Microdélices Apéro Tenebrio Salsa	210 – 240	A		120 – 135	A	
Pasta Microdélices	215 – 245	B		125 – 135	B	
Blood Worm	200 – 240	A		120 – 130	A	
Crispy Silkworms	200 – 230	A		120 – 135	A	
Crispy Small Crickets	215 – 245	A		120 – 130	A	
Worm Chocolate	215 – 235	A		80 – 95	A	
Midge Larva	200 – 225	A		125 – 135	A	
Barley Pests	220 – 245	A		125 – 135	A	
Mealworms	215 – 240	A		120 – 135	A	
Mealworm Powder	200 – 240	A		125 – 135	A	
Cricket Flour	215 – 240	A		125 – 140	A	
Maggot Powder	215 – 235	A		125 – 135	A	
Silkworm Chrysalis	200 - 230	A		130 - 135	A	

Table 2 shows the Nitrogen/Protein data obtained using helium as carrier gas while Table 3 the Nitrogen/Protein data obtained using argon as carrier gas. Each sample was analyzed five times. The data are comparable and the repeatability is more than acceptable giving in both cases a RSD% less than 2% as Official Methods requirements.

Table 2. Nitrogen/Protein data using helium carrier gas.

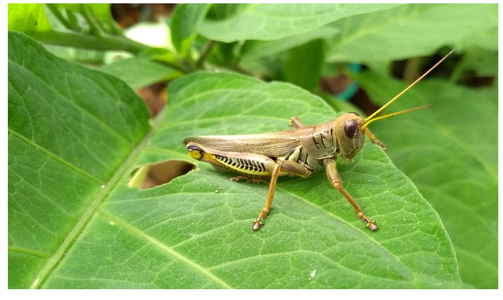
Sample Name	N%	RSD%	Protein %	RSD%
Microdélices Classic Nature Tenebrio	9.08	0.25	56.75	0.27
Microdélices Classic Nature Sigillatus	9.02	0.14	56.37	0.16
Microdélices Apéro Tenebrio Barbecue	8.49	0.12	53.08	0.12
Microdélices Apéro Tenebrio Salsa	8.78	0.35	54.89	0.33
Pasta Microdélices	3.57	0.34	22.30	0.37
Blood Worm	7.32	0.30	45.75	0.28
Crispy Silkworms	7.98	0.27	49.89	0.26
Crispy Small Crickets	8.99	0.36	56.22	0.34
Worm Chocolate	2.35	0.88	14.66	0.83
Midge Larva	7.74	0.50	48.36	0.52
Barley Pests	7.96	0.39	49.77	0.38
Mealworms	8.94	0.22	55.88	0.22
Mealworm Powder	8.66	0.82	54.08	0.80
Cricket Flour	11.04	0.27	69.04	0.27
Maggot Powder	8.70	0.37	54.37	0.36
Silkworm Chrysalis	8.71	0.53	54.44	0.55

Table 3. Nitrogen/Protein data using argon carrier gas.

Sample Name	N%	RSD%	Protein %	RSD%
Microdélices Classic Nature Tenebrio	9.03	0.48	56.45	0.48
Microdélices Classic Nature Sigillatus	9.08	0.61	56.73	0.60
Microdélices Apéro Tenebrio Barbecue	8.46	0.36	52.87	0.39
Microdélices Apéro Tenebrio Salsa	8.74	0.45	54.62	0.42
Pasta Microdélices	3.45	0.81	22.16	0.73
Blood Worm	7.35	0.85	45.96	0.83
Crispy Silkworms	7.97	0.45	49.81	0.44
Crispy Small Crickets	9.04	0.43	56.47	0.41
Worm Chocolate	2.36	0.95	14.75	0.97
Midge Larva	7.71	0.37	48.23	0.33
Barley Pests	7.97	0.55	49.81	0.56
Mealworms	9.00	0.37	56.25	0.37
Mealworm Powder	8.59	0.51	53.69	0.47
Cricket Flour	10.99	0.57	68.67	0.58
Maggot Powder	8.67	0.44	54.17	0.43
Silkworm Chrysalis	8.72	0.69	54.52	0.67

CONCLUSIONS

- The Flash*Smart* Elemental Analyzer, based on the combustion method (Dumas), offers advantages over the Kjeldahl Method for the Nitrogen/Protein determination in terms of automation, ease of use and cost per sample.
- The Flash*Smart* Elemental Analyzer, using argon as carrier gas enables to perform Nitrogen/Protein analysis in a large range of concentrations in many types of insect-based food and animal feed without matrix effect. The Nitrogen/Protein data obtained are comparable with those obtained using helium as carrier gas.
- The RSD% obtained was less than 2% of the performance requirements of the Official Methods.
- No memory effect was observed, indicating complete combustion and detection of the element independent of the sample matrix.
- The application showed that the Dumas Method meets manufacturers and laboratories requirements, including compliance to official methods.
- The Dumas Combustion method has been approved and adopted by Official Organizations such as ASBC, AOAC, AACC, AOCS, IDF, IFFO and ISO.



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TRADEMARKS/LICENSING

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