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N/Protein and CHNS determination of Insect-based Food by Flash*Smart* Elemental Analyzer

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ABSTRACT

Purpose: Nitrogen/Protein determination by Dumas combustion method (Dumas method)

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

Results: Nitrogen/Protein and CHNS 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 of food and animal feed are essential for consumers. Regulations at national level require labeling of food products with comprehensive nutritional facts, 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 has been legalized in some countries (for example France, Switzerland) and the demand has increased. The advantage of consuming insect-based food is related to the high-protein value and low fats. Also, insect-based food is easy to produce. Unfortunately, the price isn't competitive and they are more expensive than other alternatives.The precise and accurate determination of the protein amount, through the determination of nitrogen, is fundamental to achieve the nutritional quality of finished products.

The increase in the consumption of insect-based food and feed boosted the need for precise and accurate determination of the protein amount. 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 different associations (AOAC, AACC, AOCS, ASBC, IDF, ISO and IFFO). The Thermo Scientific™ Flash*Smart*™ Elemental Analyzer (Figure 1), based on the dynamic flash combustion of the sample, cope with a wide array of important requirements of laboratories such as accuracy, day by day reproducibility and high sample throughput. However, as in the last years there was significant cost increase in helium, it is necessary to test as alternative gas, argon which is readily available.

Figure 1. Thermo Scientific Flash*Smart* Elemental Analyzer.

METHOD

The 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.

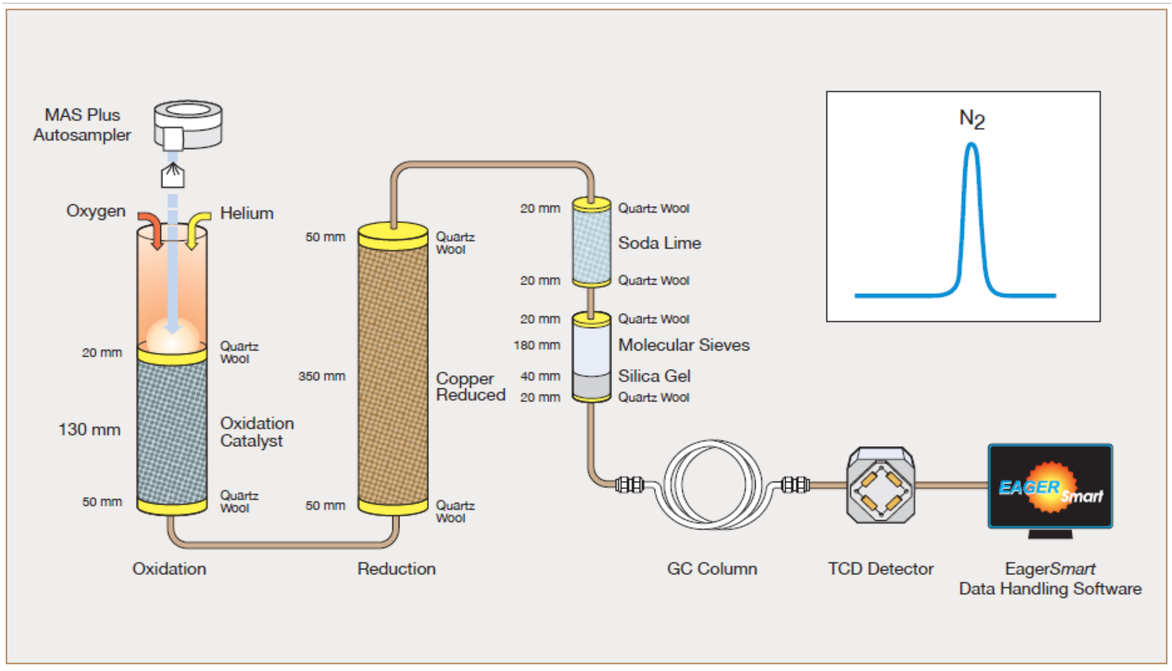

Figure 2. Nitrogen/Protein configuration.

RESULTS

Several insect-based food samples 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 samples were homogenized by a ball mill. Table 1 shows the sample weight and the standard used for calibration when helium or argon is used as carrier gas.

Table 1. Samples and standard for calibration.

Sample Name	Helium carrier gas		Argon Carrier gas	
	Sample Weight (mg)	Calibration	Sample Weight (mg)	Calibration
Microdélices Classic Nature Tenebrio	200 - 220	Nicotinamide 50-100 mg	120 - 130	Nicotinamide 50-70 mg
Microdélices Classic Nature Sigillatus	210 - 230		120 – 135	
Microdélices Apéro Tenebrio Barbecue	200 – 215		120 – 135	
Microdélices Apéro Tenebrio Salsa	210 – 240		120 – 135	
Pasta Microdélices	215 – 245		125 – 135	
Crispy Silkworms	200 – 230		120 – 135	
Crispy Small Crickets	215 – 245		120 – 130	
Worm Chocolate	215 – 235		80 – 95	
Cricket Flour	215 – 240		125 – 140	
Silkworm Chrysalis	200 - 230		130 - 135	

Table 2 shows the Nitrogen/Protein data obtained using helium as carrier gas. Table 3 shows 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
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
Cricket Flour	11.04	0.27	69.04	0.27
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
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
Cricket Flour	10.99	0.57	68.67	0.58
Silkworm Chrysalis	8.72	0.69	54.52	0.67

Finally, after an upgrade from nitrogen to CHNS configuration, a complete characterization of the samples can be performed. The data of nitrogen, carbon, hydrogen and sulfur obtained simultaneously by combustion method is shown in Table 4. The calibration was performed with 2-3 mg BBOT standard (2,5-Bis (5-ter-butyl-benzoxazol-2-yl) thiophene) using K factor as calibration method. The calibration was evaluated by the analysis of BBOT and aspartic acid as unknown. Table 4 shows the CHNS data of the samples analyzed 10 times each sample, sample weight 3-4 mg.

Table 4. CHNS data of insect food and animal feed.

Sample Name	N%	RSD%	C%	RSD%	H%	RSD%	S%	RSD%
Microdélices Classic Nature Tenebrio	9.08	0.30	49.60	0.31	7.29	0.33	0.245	0.341
Microdélices Classic Nature Sigillatus	9.02	0.29	49.83	0.27	7.37	0.25	0.320	0.262
Microdélices Apéro Tenebrio Barbecue	8.52	0.19	48.35	0.20	7.13	0.22	0.280	0.253
Microdélices Apéro Tenebrio Salsa	8.78	0.27	49.80	0.24	7.25	0.25	0.291	0.227
Pasta Microdélices	3.54	0.15	40.14	0.14	6.18	0.11	0.169	0.000
Crispy Silkworms	7.93	0.17	55.72	0.16	8.17	0.16	0.475	0.115
Crispy Small Crickets	8.92	0.18	47.23	0.17	6.95	0.14	0.385	0.184
Worm Chocolate	2.36	030	57.48	0.21	8.80	0.19	0.093	0.480
Cricket Flour	11.06	0.14	55.10	0.14	8.03	0.14	0.463	0.181
Silkworm Chrysalis	8.72	0.17	51.25	0.16	7.26	0.14	0.291	0.230

CONCLUSIONS

The Thermo Scientific 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.
Good repeatability was also obtained for CHNS determination and the nitrogen values are comparable with those obtained using the N/Protein configuration. 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, IPIFF and ISO.

ACKNOWLEDGEMENTS

We would like to thank Luca Mignani, IIS Curie-Sraffa student (Milan, Italy) for his collaboration in the analysis of the samples during his internship in Thermo Scientific OEA Demo Lab (Milan, Italy).

TRADEMARKS/LICENSING

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