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APPLICATION NOTE 42336

# Elemental Analysis: N/Protein and CHNS determination of insect-based food and animal feed by Dumas method


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**Keywords**

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## Goal

Demonstrate the performance of the elemental analyzer using helium and argon as carrier gas in alternative to the Kjeldahl method and CHNS determination of insect food and animal feed analysis.

## 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 use of insect-based food has spread for animal feed, for fishes for the most.

The increase in the consumption of insect-based food and feed boosted the need for precise and accurate determination of the protein amount. By determining nitrogen, the nutritional value of animal finished products can be obtained. For these reasons, the Dumas method (combustion method) supports the determination of nitrogen, in a faster, safer and more reliable way 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).

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The Thermo Scientific™ Flash*Smart*™ Elemental Analyzer (Figure 1), based on the dynamic flash combustion of the sample, performs nitrogen determination for insect-based food. The Flash*Smart* Elemental Analyzer meets a wide range of important requirements of laboratories such as accuracy, day by day reproducibility and high sample throughput. 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. The Flash*Smart* EA allows also the simultaneous determination of nitrogen, carbon, hydrogen and sulfur by combustion method using the same system with a specific single combustion-reduction reactor for the complete characterization of all type of matrices.

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

In this application note we show results of N/Protein using helium and argon as carrier gas, and CHNS data performed with the Flash*Smart* Elemental Analyzer.

## Methods

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.

For Nitrogen/Protein determination, after combustion, the produced gases are carried by a 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.

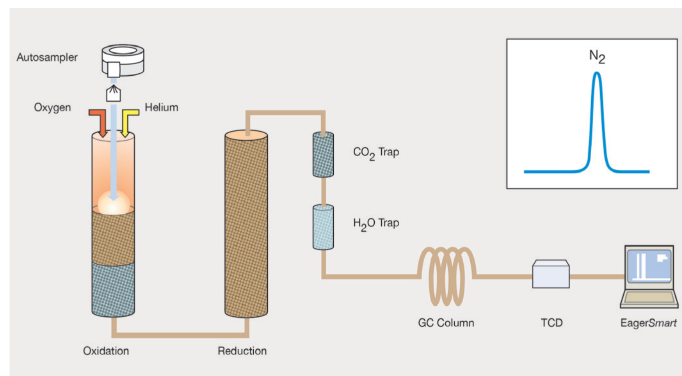


**Figure 2. Nitrogen/Protein configuration.**

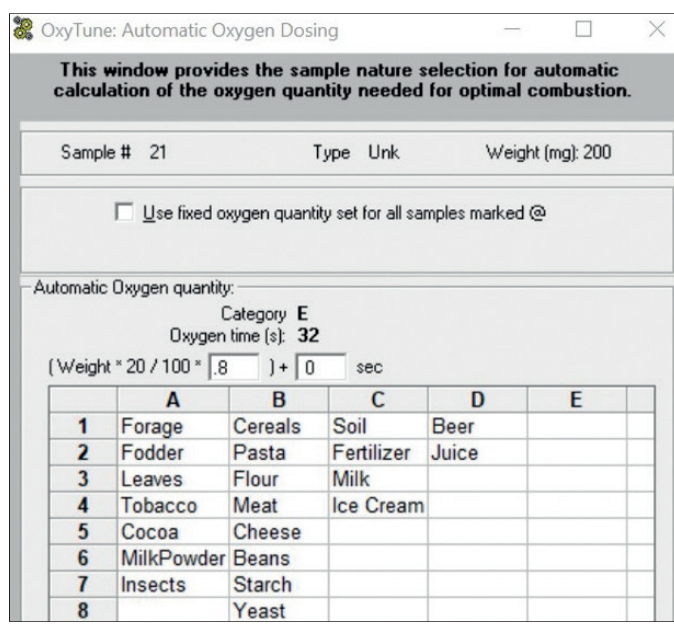


**Figure 3. OxyTune Eager*Smart* Data Handling Software window.**

For CHNS determination, after combustion, the produced gases are carried in a helium carrier gas to a layer, filled with copper. The analyte then enters the GC column, which separates the produced gases before detection by a Thermal Conductivity Detector (TCD) (Figure 4). For weight percent determination a complete report is automatically generated by the Thermo Scientific Eager*Smart* Data Handling Software and displayed at the end of the analysis.

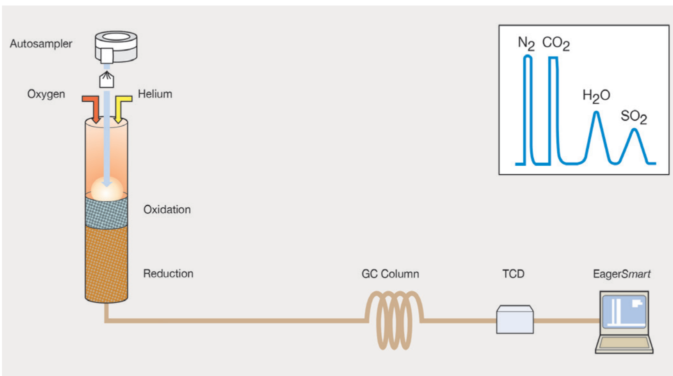


**Figure 4. CHNS configuration.**

## 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 categoy | Calibration | Sample weight (mg) | OxyTune categoy | 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 |

At last 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 |
| Blood Worm | 7.37 | 0.15 | 33.70 | 0.15 | 4.82 | 0.15 | 0.377 | 0.119 |
| 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 |
| Midge Larva | 7.96 | 0.11 | 39.17 | 0.12 | 5.86 | 0.14 | 0.438 | 0.125 |
| Barley Pests | 7.94 | 0.14 | 55.64 | 0.14 | 8.36 | 0.16 | 0.203 | 0.270 |
| Mealworms | 8.97 | 0.16 | 57.77 | 0.17 | 8.70 | 0.17 | 0.356 | 0.154 |
| Mealworm Powder | 8.63 | 0.10 | 50.72 | 0.08 | 7.54 | 0.11 | 0.286 | 0.192 |
| Cricket Flour | 11.06 | 0.14 | 55.10 | 0.14 | 8.03 | 0.14 | 0.463 | 0.181 |
| Maggot Powder | 8.73 | 0.14 | 48.07 | 0.14 | 7.07 | 0.16 | 0.480 | 0.110 |
| 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 and ISO.

## Acknowledgements

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