# Nitrogen/Protein Determination of Spirulina Algae by Dumas Method

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## ABSTRACT

**Purpose:** Nitrogen/Protein determination by Dumas combustion method.

**Methods:** Spirulina algae was analyzed through an elemental analyzer with automatic 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

Spirulina is a natural algae (cyanbacteria) powder with high protein content, with antioxidants, B-vitamins and other nutrients. It is largely made up of protein and essential amino acids, and is typically recommended to vegetarians for its high protein and iron content. The high concentration of protein and iron also makes it ideal during pregnancy, after surgery, or anytime the immune system needs a boost.

For all these benefits, the precise and accurate determination of the protein amount, through the determination of nitrogen, is fundamental to achieve the nutritional quality of this product. 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.

The Dumas Combustion method has been approved and adopted by Official Organizations such as ASBC, AOAC, AACC, AOCS, IDF, IFFO and ISO.

The Thermo Scientific™ Flash*Smart*™ Elemental Analyzer (Figure 1), 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 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 Detector) (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, for the spirulina algae 6.25 was used as protein factor. Figure 3 shows a typical chromatogram with the relative nitrogen peak.

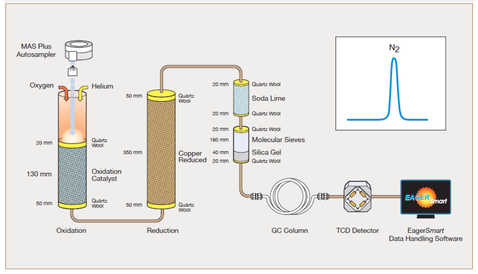


**Figure 2. Nitrogen/Protein configuration.**

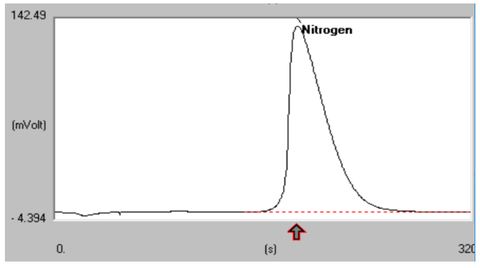


**Figure 3. Typical chromatogram.**

## RESULTS

Spirulina algae was analyzed by 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 spirulina algae sample. 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%). Table 1 shows the sequence of analysis using helium as carrier gas while Table 2 the sequence of analysis using argon as carrier gas. Spirulina algae was analyzed 10 times.

**Table 1. Sequence of analysis using helium as carrier gas.**

| Run | Sample Name | Type | Weight (mg) | N% |
|---|---|---|---|---|
| 1 | Nicotinamide | Standard | 70.2 | 22.94 |
| 2 | Nicotinamide | Standard | 95.7 | 22.94 |
| 3 | Nicotinamide | Unknown | 90.4 | 23.02 |
| 4 | Aspartic acid | Unknown | 74.5 | 10.56 |
| 5 | Spirulina algae | Unknown | 242.3 | 10.80 |
| 6 | Spirulina algae | Unknown | 260.5 | 10.85 |
| 7 | Spirulina algae | Unknown | 215.8 | 10.80 |
| 8 | Spirulina algae | Unknown | 241.3 | 10.82 |
| 9 | Spirulina algae | Unknown | 230.2 | 10.79 |
| 10 | Spirulina algae | Unknown | 222.7 | 10.84 |
| 11 | Spirulina algae | Unknown | 248.5 | 10.85 |
| 12 | Spirulina algae | Unknown | 246.8 | 10.86 |
| 13 | Spirulina algae | Unknown | 218.9 | 10.81 |
| 14 | Spirulina algae | Unknown | 219.1 | 10.80 |
| 15 | Aspartic acid | Unknown | 78.3 | 10.58 |
| 16 | Nicotinamide | Unknown | 69.9 | 22.87 |

**Table 2. Sequence of analysis using argon as carrier gas.**

| Run | Sample Name | Type | Weight (mg) | N% |
|---|---|---|---|---|
| 1 | Nicotinamide | Standard | 58.1 | 22.94 |
| 2 | Nicotinamide | Standard | 72.6 | 22.94 |
| 3 | Nicotinamide | Unknown | 68.5 | 23.00 |
| 4 | Aspartic acid | Unknown | 77.5 | 10.59 |
| 5 | Spirulina algae | Unknown | 126.4 | 10.80 |
| 6 | Spirulina algae | Unknown | 119.2 | 10.77 |
| 7 | Spirulina algae | Unknown | 129.5 | 10.82 |
| 8 | Spirulina algae | Unknown | 125.3 | 10.75 |
| 9 | Spirulina algae | Unknown | 120.4 | 10.81 |
| 10 | Spirulina algae | Unknown | 120.9 | 10.83 |
| 11 | Spirulina algae | Unknown | 119.7 | 10.74 |
| 12 | Spirulina algae | Unknown | 121.5 | 10.78 |
| 13 | Spirulina algae | Unknown | 125.1 | 10.75 |
| 14 | Spirulina algae | Unknown | 125.8 | 10.81 |
| 15 | Aspartic acid | Unknown | 53.8 | 10.49 |
| 16 | Nicotinamide | Unknown | 59.9 | 22.87 |

Table 3 shows the Nitrogen/Protein data obtained of the spirulina algae using helium and argon as carrier gas. The sample weight was 200 – 250 mg and it was analyzed 10 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 3. Nitrogen/Protein data comparison of spirulina algae.**

| | Helium carrier gas | | Argon carrier gas | |
|---|---|---|---|---|
| | N% | Protein % | N% | Protein % |
| | 10.80 | 67.50 | 10.80 | 67.47 |
| | 10.85 | 67.81 | 10.77 | 67.31 |
| | 10.80 | 67.49 | 10.82 | 67.65 |
| | 10.82 | 67.63 | 10.75 | 67.18 |
| | 10.79 | 67.44 | 10.81 | 67.56 |
| | 10.84 | 67.75 | 10.83 | 67.69 |
| | 10.85 | 67.82 | 10.74 | 67.13 |
| | 10.86 | 67.88 | 10.78 | 67.37 |
| | 10.81 | 67.56 | 10.75 | 67.20 |
| | 10.80 | 67.52 | 10.81 | 67.57 |
| **Average** | 10.82 | 67.62 | 10.79 | 67.41 |
| **Std.Dev.** | 0.0257 | 0.1610 | 0.0324 | 0.2040 |
| **RSD%** | 0.24 | 0.25 | 0.30 | 0.30 |

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.

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 3 shows the CHNS data of the spirulina algae analyzed 10 times weighing 3-4 mg.

**Table 4. CHNS data of spirulina algae.**

| | N% | C% | H% | S% |
|---|---|---|---|---|
| | 10.79 | 47.22 | 6.89 | 0.612 |
| | 10.80 | 47.22 | 6.94 | 0.619 |
| | 10.80 | 47.20 | 6.88 | 0.612 |
| | 10.83 | 47.28 | 6.91 | 0.619 |
| | 10.80 | 47.30 | 6.88 | 0.619 |
| | 10.80 | 47.20 | 6.88 | 0.615 |
| | 10.82 | 47.17 | 6.89 | 0.609 |
| | 10.83 | 47.31 | 6.91 | 0.613 |
| | 10.80 | 47.26 | 6.92 | 0.615 |
| | 10.81 | 47.19 | 6.91 | 0.607 |
| **Average** | 10.81 | 47.23 | 6.90 | 0.614 |
| **Std.Dev.** | 0.0140 | 0.0490 | 0.0202 | 0.0042 |
| **RSD%** | 0.13 | 0.10 | 0.29 | 0.69 |

## CONCLUSIONS

- The 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, ease of use and cost per sample.
- The Flash*Smart* Elemental Analyzer, using argon as carrier gas, enables to analyze high Nitrogen/Protein content 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.
- The application showed that the Dumas Method meets manufacturers and laboratories requirements, including the 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.
- The Flash*Smart* EA allows the complete characterization of the sample using the CHNS configuration. The data obtained shows a good repeatability of the four elements. The nitrogen value is comparable with those obtained using the Nitrogen/Protein configuration.

## TRADEMARKS/LICENSING



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