

# Elemental Analysis: N/Protein and CHNS determination of spirulina algae by Dumas method

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## Goal

To assess the performance of the elemental analyzer for the N/Protein determination of spirulina algae using helium and argon as carrier gas in alternative to the Kjeldahl method and the CHNS determination.

## Introduction

Spirulina is a natural algae (cyanobacteria) 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™ FlashSmart™ 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 FlashSmart 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 FlashSmart EA. The FlashSmart 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 this type of matrix.

In this note we assess the performance of the elemental analyzer for the N/Protein determination of spirulina algae using helium and argon as carrier gas in alternative to the Kjeldahl method and the CHNS determination.



Figure 1. Thermo Scientific FlashSmart Elemental Analyzer.

## Methods

The FlashSmart EA 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<sub>2</sub> and H<sub>2</sub>O traps, a GC column and finally detected by a Thermal Conductivity Detector (TCD Detector) (Figure 2).

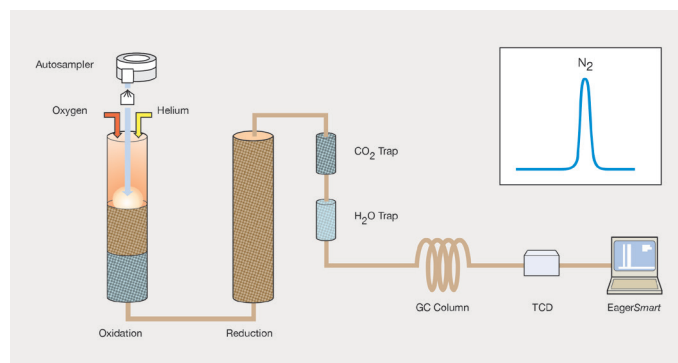


Figure 2. Nitrogen/Protein configuration.

A complete report is automatically generated by the Thermo Scientific™ EagerSmart™ 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.

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 3). For weight percent determination a complete report is automatically generated by the Thermo Scientific™ EagerSmart Data Handling Software and displayed at the end of the analysis.

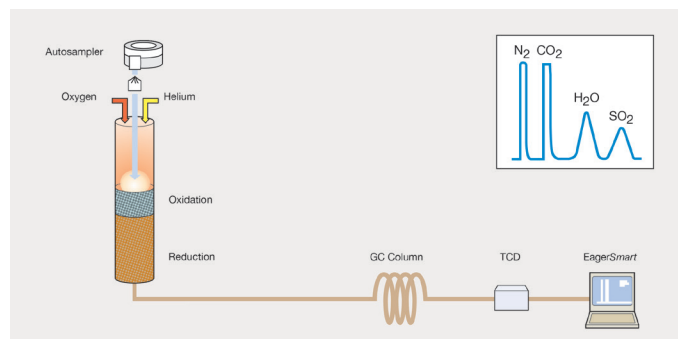


Figure 3. CHNS configuration.

## Results

For N/Protein determination, spirulina algae was analyzed by the FlashSmart 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. Using helium carrier gas, the sample was weighed at 200 – 250 mg while using argon carrier gas, the sample was weighed at 120 – 125 mg. The sample was analyzed 10 times. The data are comparable and the repeatability is more than acceptable giving in both cases a RSD% less than 2%, falling within 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
<b>Average</b>	10.82	67.62	10.79	67.41
<b>Std.Dev.</b>	0.0257	0.1610	0.0324	0.2040
<b>RSD%</b>	0.24	0.25	0.30	0.30

At last the data of the simultaneous CHNS determination 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 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
<b>Average</b>	10.81	47.23	6.90	0.614
<b>Std.Dev.</b>	0.0140	0.0490	0.0202	0.0042
<b>RSD%</b>	0.13	0.10	0.29	0.69



## Conclusions

The Thermo Scientific FlashSmart 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 FlashSmart 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 FlashSmart 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.

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