Nitrogen/Protein determination of infant food by the Thermo Scientific FlashSmart Elemental Analyzer using helium or argon as carrier gases

Introduction
The nutritional composition of infant food plays a very important role in food industries for research and quality control purposes. Food market globalization requires accurate control of product characteristics to protect commercial value, to safeguard consumer health, and to maintain manufacturer reputation. The selection of infant food products available in the market is very wide, and consumers’ decisions depend on a set of information that includes quality valuation. New regulations regarding all processed food and most raw foods include a series of tests aimed at determining food contents and their contribution to a healthy diet for infants and children. Official regulations establish protein content and labeling requirements which enable consumers to make quality comparisons.

Goal
To demonstrate the performance of the Thermo Scientific FlashSmart Elemental Analyzer for food quality and labeling purposes, while showing compliance to international standards requirements.

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Keywords
Argon, Combustion, Food Quality, Infant Food, Labeling, Nitrogen, Protein
One of the tests used in the production process is the determination of protein content through nitrogen analysis. It is very important to have an accurate and precise technique for periodically performing this test, and an automated technique that provides fast analysis, excellent reproducibility, and avoids the use of toxic chemicals is ideal. An alternative to the classical Kjeldahl method, based on the Dumas (combustion) method, has been developed and is approved by different organizations.

The Thermo Scientific™ FlashSmart™ Elemental Analyzer (Figure 1), based on the dynamic combustion method (Dumas method), provides rapid and automated nitrogen determination without use of hazardous chemicals and offers advantages in precision over traditional methods. The FlashSmart Elemental Analyzer allows runs at both high and low nitrogen levels with no need to change configurations and without matrix effects.

However, due to a possible worldwide shortage and increase in the cost of helium, the FlashSmart Analyzer can work with an alternative gas, argon which is readily available.

Methods
The Elemental Analyzer operates according to the dynamic flash combustion (modified Dumas method) of the sample. Powdered samples were weighed in tin containers and liquid samples were adsorbed on the inert material Chromosorb, then weighed in tin containers. Both were introduced into the combustion reactor with oxygen via the Thermo Scientific™ MAS Plus Autosampler. After combustion, the produced gases were carried by a helium or argon flow to a second reactor filled with copper. Then they were swept through CO₂ and H₂O traps, a GC column, and were finally detected by a thermal conductivity detector (Figure 2). A complete report is automatically generated by the Thermo Scientific™ EagerSmart™ Data Handling Software and displayed at the end of the analysis. Using the nitrogen data obtained and a protein factor, the software automatically calculates the protein content.

Table 1 shows the analytical conditions using helium and argon as carrier gases.

Table 1. Analytical conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Helium Carrier Gas</th>
<th>Argon Carrier Gas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combustion Reactor Temperature</td>
<td>950 °C</td>
<td>950 °C</td>
</tr>
<tr>
<td>Reduction Reactor Temperature</td>
<td>840 °C</td>
<td>840 °C</td>
</tr>
<tr>
<td>Oven Temperature</td>
<td>50 °C</td>
<td>50 °C</td>
</tr>
<tr>
<td>Carrier Gas Flow</td>
<td>140 mL/min</td>
<td>60 mL/min</td>
</tr>
<tr>
<td>Reference Gas Flow</td>
<td>100 mL/min</td>
<td>60 mL/min</td>
</tr>
<tr>
<td>Total Run Time</td>
<td>400 sec</td>
<td>600 sec</td>
</tr>
<tr>
<td>Sample Delay</td>
<td>10 sec</td>
<td>10 sec</td>
</tr>
<tr>
<td>Oxygen Flow</td>
<td>300 mL/min</td>
<td>300 mL/min</td>
</tr>
<tr>
<td>OxyTune Category</td>
<td>@ 30 sec: aspartic acid, Cat. A: samples 1, 3, 4, 5, 6, 7, 8, 9, 10 and 11.</td>
<td>@ 30 sec: aspartic acid, @ 30 sec: all powder samples</td>
</tr>
<tr>
<td></td>
<td>Cat. C: urea/water solution and liquid samples 12 and 13</td>
<td>Cat. C: urea/water solution and liquid samples 12 and 13</td>
</tr>
<tr>
<td></td>
<td>Category E (d = 1.4) for sample 2</td>
<td>Category E (d = 1.4) for sample 2</td>
</tr>
<tr>
<td>GC Column</td>
<td>0.5 m, installed outside the oven</td>
<td>1 m, installed in the oven</td>
</tr>
</tbody>
</table>

Note: The EagerSmart Data Handling Software provides a new option AGO (Argon Gas Option) that allows the user to manage the flow of argon gas during the run.
Results
To evaluate the performance of the system using helium and argon as carrier gases, several infant food samples (powders and liquids) with different nitrogen concentrations were chosen.

Table 2 shows the sample information and Table 3 shows the standards used for calibration and the weight of each sample. K factor was used as the calibration method in all cases.

<table>
<thead>
<tr>
<th>Ref. No.</th>
<th>Matrix Type</th>
<th>Consuming Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Milk based powder, sucrose free</td>
<td>1–3 years</td>
</tr>
<tr>
<td>2</td>
<td>Milk based powder</td>
<td>From birth onward</td>
</tr>
<tr>
<td>3</td>
<td>Milk based powder</td>
<td>1–3 years</td>
</tr>
<tr>
<td>4</td>
<td>Milk based powder</td>
<td>1–3 years</td>
</tr>
<tr>
<td>5</td>
<td>Milk based powder</td>
<td>3 years onward</td>
</tr>
<tr>
<td>6</td>
<td>Milk based powder</td>
<td>1–8 years</td>
</tr>
<tr>
<td>7</td>
<td>Milk based powder</td>
<td>6–12 months</td>
</tr>
<tr>
<td>8</td>
<td>Milk based powder, sucrose free</td>
<td>0–6 months</td>
</tr>
<tr>
<td>9</td>
<td>Milk based powder</td>
<td>1–10 years</td>
</tr>
<tr>
<td>10</td>
<td>Milk based powder</td>
<td>From birth onward</td>
</tr>
<tr>
<td>11</td>
<td>Milk based powder, sucrose free</td>
<td>From birth onward</td>
</tr>
<tr>
<td>12</td>
<td>Milk based powder</td>
<td>From birth onward</td>
</tr>
<tr>
<td>13</td>
<td>Milk based powder</td>
<td>From birth onward (premature babies)</td>
</tr>
</tbody>
</table>

Table 2. Sample information.

<table>
<thead>
<tr>
<th>Helium Carrier Gas</th>
<th>Standard</th>
<th>Sample</th>
<th>Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid (10.52 N%) 50-100 mg</td>
<td>1</td>
<td>200–220</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>210–220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>220–230</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>200–220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>170–180</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>200–220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>190–200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>200–210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>200–220</td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td>200–240</td>
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<tr>
<td>11</td>
<td>200–220</td>
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<td>12</td>
<td>180–220</td>
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<td></td>
</tr>
<tr>
<td>13</td>
<td>180–220</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Argon Carrier Gas</th>
<th>Standard</th>
<th>Sample</th>
<th>Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid (10.52 N%) 50-70 mg</td>
<td>1</td>
<td>110–115</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>110–115</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>110–115</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>110–115</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>110–115</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
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<td>7</td>
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<td>11</td>
<td>110–115</td>
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<tr>
<td>12</td>
<td>220–270</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>220–270</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Urea/water solution (0.46 N%) 160-200 mg | 1 | 200–220 |
| 2 | 210–220 |
| 3 | 220–230 |
| 4 | 200–220 |
| 5 | 170–180 |
| 6 | 200–220 |
| 7 | 190–200 |
| 8 | 200–210 |
| 9 | 200–220 |
| 10 | 200–240 |
| 11 | 200–220 |
| 12 | 180–220 |
| 13 | 180–220 |

| Urea/water solution (0.46 N%) 260-270 mg | 1 | 110–115 |
| 2 | 110–115 |
| 3 | 110–115 |
| 4 | 110–115 |
| 5 | 110–115 |
| 6 | 110–115 |
| 7 | 110–115 |
| 8 | 110–115 |
| 9 | 110–115 |
| 10 | 110–115 |
| 11 | 110–115 |
| 12 | 220–270 |
| 13 | 220–270 |
Table 4 shows the nitrogen/protein data obtained from samples listed in Table 3 by using helium and argon as carrier gases. Each sample was analyzed in triplicate. The protein factor used to calculate the protein content through the nitrogen value was 6.38.

Table 4. Nitrogen/protein data using helium and argon as carrier gas.

<table>
<thead>
<tr>
<th>Sample Ref. No.</th>
<th>Helium as carrier gas</th>
<th>Argon as carrier gas</th>
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<tbody>
<tr>
<td></td>
<td>N%</td>
<td>Protein %</td>
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<tr>
<td>1</td>
<td>2.34</td>
<td>14.90</td>
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<tr>
<td></td>
<td>2.33</td>
<td>14.89</td>
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<td></td>
<td>2.34</td>
<td>14.92</td>
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<td>2</td>
<td>2.23</td>
<td>14.26</td>
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<td>14.26</td>
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<td>3</td>
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<td>2.94</td>
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<tr>
<td></td>
<td>0.450</td>
<td>2.87</td>
</tr>
</tbody>
</table>

Table 5 presents a summary of the protein averages comparing data from helium gas, argon gas, and from the Kjeldahl analysis. The protein % determined by Kjeldahl analysis was performed at least 3–4 times for every sample, and includes the acceptable range for results. The data comparison demonstrates the performance of the instrument using both gases.
Conclusions

All data shown were obtained with excellent repeatability, and no matrix effect was observed when changing the sample. Good repeatability was obtained with the FlashSmart EA Nitrogen Analyzer using argon as carrier gas, and data from argon and helium were comparable. The data is also comparable with data obtained by the Kjeldahl method.

As a complete automatic system, the FlashSmart Elemental Analyzer can analyze nitrogen in a wide content range, in solid and liquid samples, and without the use of sample digestion or the toxic chemicals used in traditional methods.

Acknowledgement

Thanks to the Ministry of Health Laboratory, Haifa, Israel, for procuring the wide range of infant food samples analyzed by the FlashSmart Elemental Analyzer.

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