Elemental Analysis: Nitrogen/Protein determination in starch by flash combustion in alternative to Kjeldahl method

Author: Liliana Krotz, Thermo Fisher Scientific, Milan, Italy

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Goal

To assess the performance of the elemental analyzer for N/Protein determination for low and trace nitrogen content in starch and CHNS determination.

Introduction

As starch is the most common carbohydrate in the human diet (being contained in in large amounts in staple food), and since it is largely used for diverse industrial uses, its elemental content must be monitored and tested for quality control.

Pure starch is a white, tasteless and odorless powder. Starch is largely used in the food industry as food additive, modified starches and sugars. For the starch refining process and from a food safety point of view, its protein content is used as an important parameter. As additive for food processing, food starches are typically used as thickeners and stabilizers in foods, such as puddings, custards, soups, sauces, gravies, pie fillings, and salad dressings, and are exceptional binders in processed meats.

For the production of ethanol in the manufacturing of beer, whisky and biofuel, starch is converted into sugars. Many of the sugars used in processed foods are produced by using starch. Mixing starches in warm water produces a paste, which can be used as a thickening, stiffening or gluing agent.

Starch is also used in industries other than food: for example as an adhesive in the papermaking process, as additive before ironing, as an excipient, as tablet disintegrant, and as binder for pharmaceutical uses.

In the production process, the protein content of starch, calculated through the nitrogen determination, is periodically monitored, and tested for quality control. Considering that starch is used also in the preparation of animal feed, the determination of N/Protein is critical. Therefore, it is very important to have an automated method allowing the analysis of N/Protein with an excellent reproducibility.

The Dumas method (combustion method) allows faster, safer, and more reliable determination of nitrogen than the traditional Kjeldahl method and has been approved by different associations.

The Thermo Scientific™ Flash*Smart* ™ Elemental Analyzer (Figure 1), based on the dynamic flash combustion of the sample, meets a wide array of requirements of laboratories such as stability, accuracy, day by day reproducibility and high sample throughput.

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

The Flash*Smart* Elemental Analyzer provides rapid and automated nitrogen determination without the use of hazardous chemicals, offering advantages over traditional methods. The Flash*Smart* EA allows analyses of high and low nitrogen levels with no configurational change and without matrix effect. The protein content is calculated automatically using the dedicated conversion factor in the Thermo Scientific™ Eager*Smart*™ Data Handling Software.

The Flash*Smart* EA also allows 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.

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.

For nitrogen determination, after combustion, the produced gases are carried by a helium 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 Eager*Smart* Data Handling Software and displayed at the end of the analysis. The Eager*Smart* Data Handling Software controls all analytical parameters of the instrument including the oxygen flow and the timing of oxygen injection. It automatically calculates the amount of oxygen, relative to the sample matrix and sample weight, through the dedicated Thermo Scientific™ OxyTune Function (patented) 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.

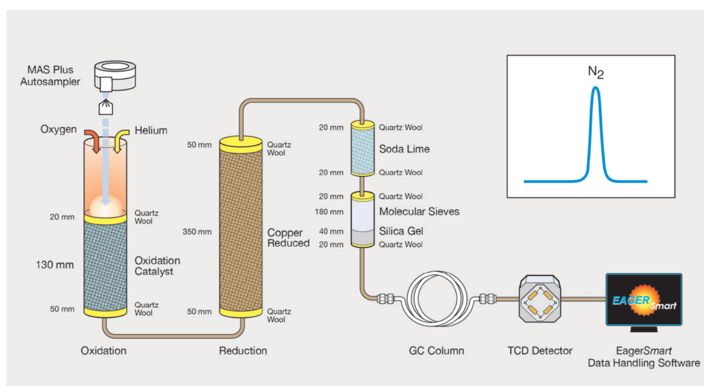

Figure 2. Nitrogen configuration.

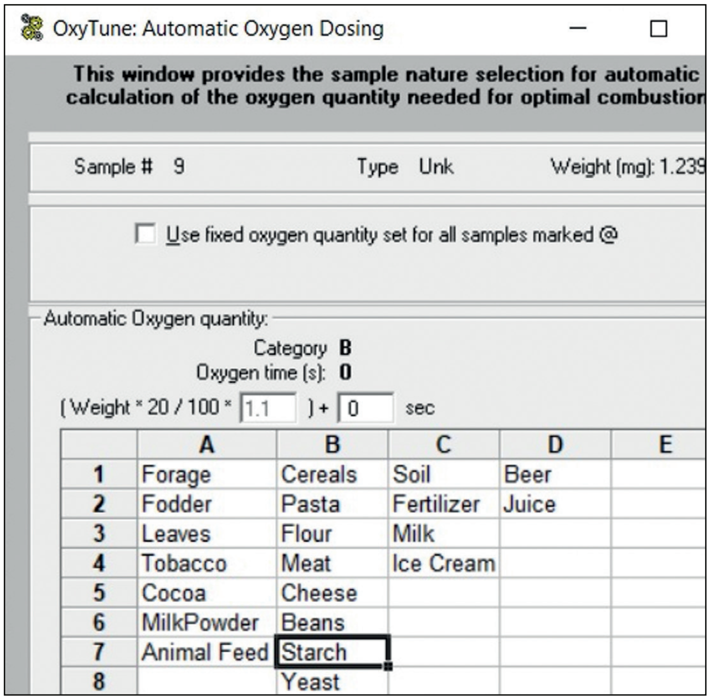

Figure 3. OxyTune Eager*Smart* Data Handling Software window.

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 Eager*Smart* Data Handling Software and displayed at the end of the analysis.

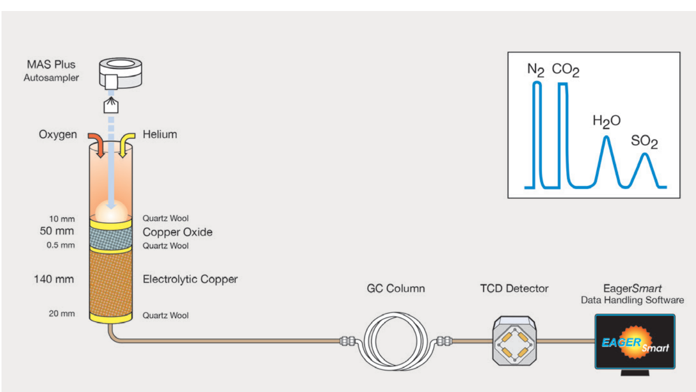

Figure 4. CHNS configuration.

Results

The starch samples analyzed were chosen on basis of the different nitrogen content. The calibration of the Analyzer was performed with 10–12 mg of atropine using K factor as calibration method. Due to the low nitrogen content present in starch samples, the blank was evaluated by the analysis of an empty tin container. The starch sample weight was about 150–250 mg.

Table 1 shows the nitrogen results obtained of starch samples at 0.25–0.35 N% analyzed in triplicate. While Table 2 shows the N/Protein data obtained of starch and slurry starch samples at trace level (100–500 ppm N) analyzed in triplicate. Slurry starch is a combination of starch (usually corn starch, flour, potato starch or arrowroot) and cold water which is mixed together and used to thicken a soup or sauce. If the starch is solely added directly to a hot liquid, the starch granules cannot disperse easily and clumps form. Once mixed with water, the slurry can be added directly to the hot liquid.

Table 1. Nitrogen data of starch samples.

Sample	N%	RSD%
1	0.2531 0.2558 0.2561	0.65
2	0.3963 0.3991 0.3940	0.64
3	0.3589 0.3563 0.3532	0.80
4	0.3029 0.3012 0.3049	0.61

Table 2. N/Protein data of starch and slurry starch samples at trace level.

Sample	N ppm	Protein %	RSD%
Starch 1	124 126 123	0.0775 0.0789 0.0768	1.38
Starch 2	162 167 164	0.1015 0.1046 0.1025	1.54
Starch 3	177 179 178	0.1107 0.1120 0.1115	0.59
Starch 4	351 350 352	0.2191 0.2185 0.2202	0.39
Starch 5	371 366 370	0.2322 0.2285 0.2312	0.83
Starch 6	380 382 387	0.2378 0.2387 0.2419	0.89
Starch 7	404 404 403	0.2528 0.2525 0.2519	0.18
Starch 8	476 474 474	0.2975 0.2962 0.2962	0.25
Starch 9	484 483 484	0.3024 0.3016 0.3024	0.15
Starch 10	546 551 548	0.3409 0.3445 0.3426	0.53
Slurry starch 1	146 150 148	0.0912 0.0937 0.0925	1.35
Slurry starch 2	166 165 162	0.1038 0.1029 0.1012	1.29

At last the data of nitrogen, carbon, hydrogen and sulfur obtained simultaneously by combustion method is shown in Table 3. 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 samples analyzed five times (sample weight 3–4 mg).

Table 3. CHNS data of a starch sample.

N%	RSD%	C%	RSD%	H%	RSD%	S%	RSD%
2.53 2.52 2.54 2.52 2.53	0.33	31.01 30.85 31.00 30.96 30.97	0.20	5.40 5.45 5.42 5.37 5.31	1.00	0.399 0.396 0.391 0.392 0.398	0.90

Conclusions

For nitrogen determination of starches, the Thermo Scientific Flash*Smart* Elemental Analyzer, based on the combustion method (Dumas), offers advantages over the Kjeldahl Method in terms of automation, ease of use and cost per sample. Differently from the traditional methods, the Flash*Smart* Elemental Analyzer doesn't require the use of toxic chemicals.

For N/Protein and CHNS determination, good repeatability was obtained, and no memory effect was observed, indicating complete combustion and detection of the element independent of the element content.

With the Flash*Smart* Analyzer laboratories can analyze nitrogen in a wide range from high to trace nitrogen content.

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

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