Case study: Dumas nitrogen determination

Fast and cost-efficient nitrogen / protein analysis for starch

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1. Introduction and presentation of problem

Due to their manufacture, starch products only contain very low concentrations of proteins. Therefore, satisfactory analysis has only been possible to date using high sample weights and long analysis times. With its innovative detection method and fast and complete combustion, DUMATHERM® from C. Gerhardt, with its innovative detection method and fast and complete combustion for the detection of Nitrogen by Dumas, provides an interesting alternative to the currently still common Kjeldahl method.

1.1 Use of starch and starch products

Starches and their derivatives are widely used in the food industry in the manufacture of confectionery, baked goods, milk products and particularly beverages in the form of starch-based sugars (mainly glucose syrup, dextrose and isoglucose). Due to the properties of the starch as modifiable polymer and its composition of fermentable sugar units, starch is also widely used as renewable raw material in the chemicals industry.[1] The many applications explain the high demand for starch-based products and the simultaneous requirement for fast and reliable analysis.

1.2 Starch production

In our part of the world, starch is usually obtained from potatoes or cereals. However, starch is also obtained from numerous other plants, of which rice (broken rice from the rice husking factories) and maize are particularly important in addition to wheat and potatoes.

Internationally, manioc (tapioca) is still an important starch resource. The extraction is performed by washing out the starch from the plant parts using a saline solution.[2]

2. Protein analysis in starch

Due to the low concentration, proteins in starch can only be quantitatively verified with great difficulty. For the DIN method according to Kjeldahl[3], big sample sizes (approx. 10 g) are weighed, digested with sulphuric acid and then subjected to further analysis steps. Strong foaming of the samples at the beginning of Kjeldahl is problematic for heating up the digestion mixture quickly to 400 °C. Large sample tubes and long digestion times are absolutely necessary. The consequence is low sample throughput per working day.

Table 1: Protein contents in starch and sample weights in Kjeldahl digestion[3]

Sample type	Typical sample weights [g]	Theoretical content [%] Protein	Theoretical content [%] Nitrogen
Potato starch	5–10	0.08 – 0.90	0.0128-0.1441
Maize starch	5–10	0.24 – 0.63	0.0384-0.1008
Rice starch	5–10	0.76	0.1216
Tapioca starch	5–10	0.05 – 0.87	0.0080-0.1392
Wheat starch	5–10	0.21 – 0.50	0.0368-0.0877

Table 1 shows the expected protein contents in various starches. It can be seen that Nitrogen / Protein analysis in Starch is almost a trace analysis using multiplication of N by the protein factor (e.g. 6.25 for maize and potato products or 5.7 in wheat). With a high sample weight in the Kjeldahl method of approx. 10 g, there is only 1 mg N (for 0.01% N content) in absolute terms. With Dumas combustion using 100-300 mg, there is only 0.01 mg nitrogen in absolute terms for the detection during analyses.

The following official methods have been used for comparison with the results obtained in this article:

- EN ISO 3188:1994 Starches and derived products - Determination of nitrogen content by the Kjeldahl method - Titrimetric method (ISO 3188:1978); Issue date (German version): 10/1994
- AOAC 992.23, Crude Protein in Cereal Grains and Oilseeds
- ICC Standard method No. 167, 2000.

However, expected precision values are not defined in the above so the Dumas method DIN EU 16634, „Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content" has been used.

2.1 Combustion analysis of starch according to the Dumas principle

Only the DUMATHERM® analyser from C. Gerhardt shown in **Figure 1** was used for the analyses presented here.

Figure 1: DUMATHERM® from C. Gerhardt with pneumatic autosampler and PC controller

2.2 Theory: combustion method for nitrogen / protein analysis

2.2.1 Sample feeding and combustion

While the acid digestion according to the Kjeldahl method destroys the sample in a rather rustic way, combustion provides a somewhat more elegant and time saving alternative. The sample is oxidised in a controlled way and the resulting gaseous products are subjected to further analysis. The objective is the complete conversion of the sample into the primary products carbon dioxide, water and nitrogen dioxide (**equation 1**) without the secondary products carbon monoxide (CO) and nitrogen monoxide (NO) being produced by incomplete combustion.

$$(CHNO)_{(s)} + O_{2\,(g)} = CO_{2\,(g)} + H_2O_{(g)} + NO_{2\,(g)} \quad (1)$$

As air has a nitrogen content far in excess of 70 %, the key to success here is effective sealing against the ambient air in combination with an efficient purging function.
An efficient autosampler is needed so that interaction of the sample and air is ruled out. **Figure 2** shows two such models.

Figure 2: Autosampler with purge function - a purge chamber is continuously purged with helium before the sample is mechanically or manually fed to the combustion by the slider.

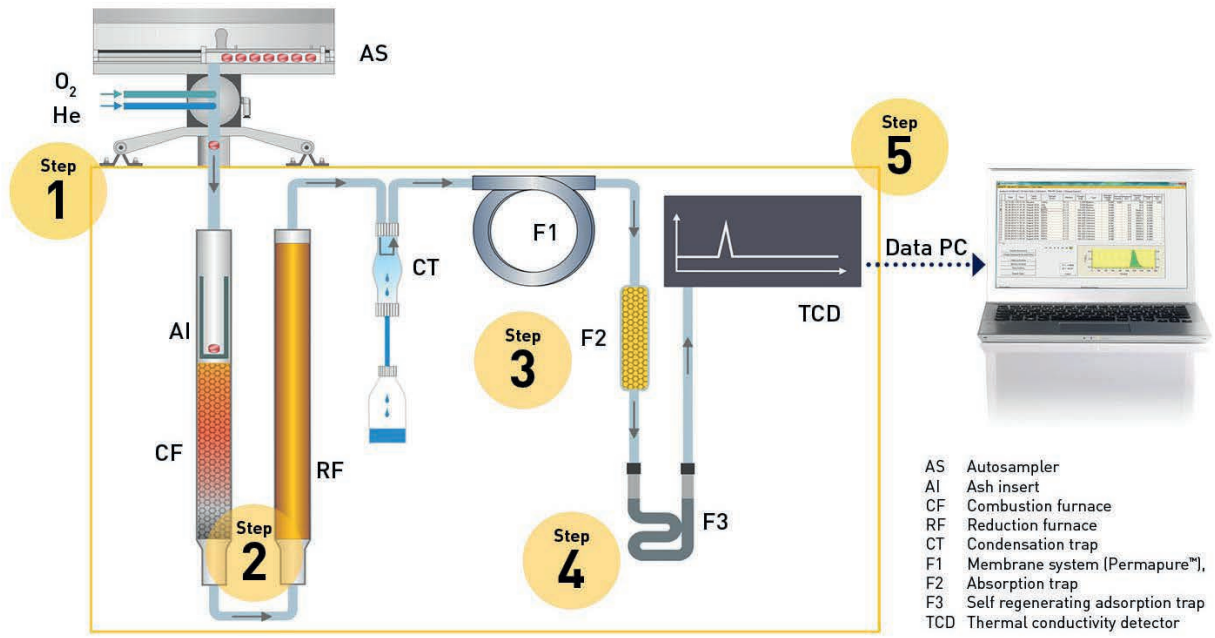

Figure 3

Modern combustion apparatus use helium technology to expel atmospheric nitrogen whilst achieving the greatest possible precision of analysis.

Figure 3 shows in detail an example of such an analysis on the DUMATHERM® from C. Gerhardt. Based on the classic principle of combustion analysis according to Jean Dumas[3] , almost maintenance-free operation is possible with modern control software.

The pneumatic autosampler (AS) feeds the solid or liquid samples (shown as red dots) packed in tin foil to the combustion furnace (CF). At the same time, the helium gas flow is changed over to oxygen to initiate the combustion. The sample incinerates and the ash produced is collected in the ash insert (AI). The gaseous combustion products react completely in the presence of catalysts to the required oxides and are transported through the machine by a helium flow after combustion. The nitrogen oxides are first reduced to elementary nitrogen in the reduction reactor (RF) while the secondary products, water and carbon dioxide, are separated in special traps (F1 to F3). A gas flow consisting of helium and nitrogen remains, from which the nitrogen can be measured using a thermal conductivity detector. Computer-control enables simultaneous evaluation of the analysis data.

2.2.2 Detection and evaluation

For ultra low level nitrogen analysis – particularly in starch application – helium is recommended as carrier gas as it is the only gas which shows very different thermal conductivity in comparison with nitrogen, **Table 2**.

Table 2: Thermal conductivities of various gases at 300 K[6]. The greater the difference in the thermal conductivity values the better the detection capability and the precision of the machine.

Gas name	$\lambda_{300\ K}$, W/m · K
Helium, He	156.7
Carbon dioxide, CO_2	16.8
Nitrogen, N_2	26.0
Carbon monoxide, CO	16.8

Helium as carrier gas and nitrogen as analyte are the ideal combination as a reference gas flow in the detector (as used in classic detectors based on a Wheatstone bridge) is unnecessary.

As only minimal amounts of nitrogen are present in starch samples, the use of helium as carrier gas is therefore crucial for the success of the analysis.

2.3 Practice: Performing a combustion analysis

In addition to the actual analysis by the DUMATHERM®, other points are still essential for good and reproducible performance:

- Sample preparation
- Sample weight
- Calibration of the detector
- Quality assurance by the user
- Selecting measurement conditions correctly
- Assessment and evaluation of the results

Tin foil is used in sample weighing and sample balls are made from this which are then kept in the sample tray until analysis.

The foil wrapped samples are placed in the sample tray and the autosampler is then closed with the cover plate before analysis (**Figure 4**).

Why do we use tin foil?

Tin combusts strongly exothermic to tin(IV)oxide. The energy released during that process supports the full combustion of the sample.

As an alternative, nitrogen free weighing paper could be used to pack the samples allthough additional amounts of ash from the paper must be considered then.

2.4 New type of calibration for starch analysis using DUMATHERM®

A new starch application has been developed for the DUMATHERM®. A key component for success here is the calibration for determining the very small nitrogen peak areas of starch samples.

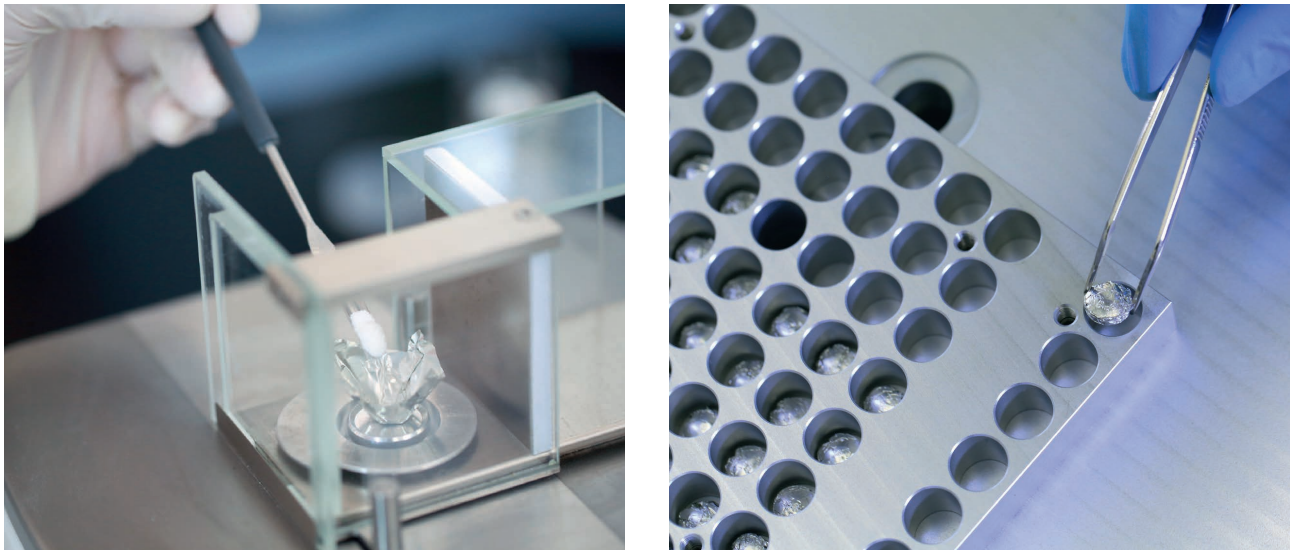

Figure 4: Sample weighing in tin foil (picture on the left) and placing samples in the sample tray until sample feeding in the autosampler (picture on the right).

Calibration which completely covers the signal levels of the unknown samples must be used for the calculation of the analysis results. Signal levels of approx. 150 - 500 mVs have been achieved for the sample weights of approx. 150 mg. As the signal levels are at the absolute detection limit, multistage calibration with a solid standard as well as with a liquid standard is recommended. **Figure 5** shows such a calibration curve, extended by measuring points at the top with the solid standard EDTA.

For the analyses of the unknown samples, standard conditions were initially used, i.e. approx. 100 - 200 mg sample weight, oxygen dosing at 400 ml/min, 990 °C combustion temperature.

The following **Figures 6 and 7** show analysis results using the examples of potato starch and wheat starch.

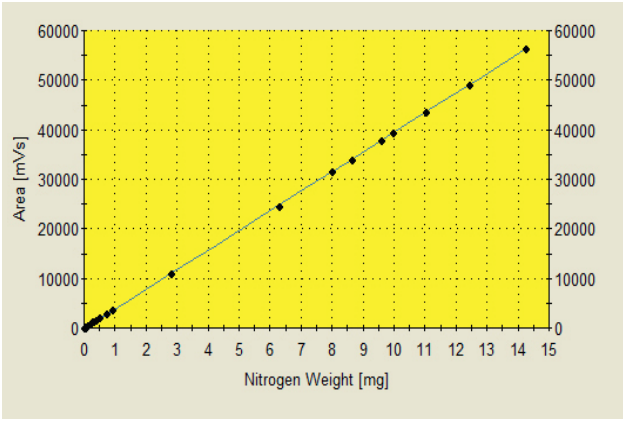

Figure 5: THAM (Tris(Hydroxymethyl) aminomethane) and EDTA (ethylene diamine tetraacetic acid), with 9.57 % N) calibration for the DUMATHERM® with a total of 20 measuring points

Dumatherm Nitrogen / Protein Analyser

Serial Number : 159 Submitter:
Software Version: DUMATHERM MANAGER V2.06E Operator: Adamek

Date	Time	Sample name	Weight [mg]	Standard name	Category	Protein factor	Peak Area [mV*s]	N Weight [mg]	Nitrogen [%]	Protein [%]
19.03.2012	11:15:17	3288	99,487		A 1,2	6,25	1,256E+02	1,743E-02	0,018	0,109
19.03.2012	11:26:23	3288	151,112		A 1,2	6,25	1,622E+02	2,679E-02	0,018	0,111
23.03.2012	12:46:49	3288	149,531		A 1,2	6,25	1,896E+02	2,635E-02	0,018	0,110
03.04.2012	10:16:56	3288	199,620		A 1,2	6,25	1,959E+02	3,527E-02	0,018	0,111
23.04.2012	09:52:04	3288	151,654		A 1,2	6,25	1,618E+02	2,651E-02	0,018	0,109

Average	0,018	0,110
Standard Deviation	0,000	0,00
RSD [%]	0,568	0,55

Calibration # : 46 (Cubic, With Zero)
Analysis Conditions for Method : Method_1
Sample Table : Analytische Studien

Temperatures:
Combustion Reactor 989 °C
Reduction Reactor 649 °C
Degassing Oven 299 °C

Times:
Sample Delay 5 s
Sample Stop 9 s
Run Time Auto

Flow Rates:
He I 195,3 sccm
He II 199,9 sccm
O_2 400,1 sccm

Figure 6:
Analysis results for potato starch with varying sample weights from 100 – 200 mg.

Dumatherm Nitrogen / Protein Analyser

Serial Number : 159 Submitter:
Software Version: DUMATHERM MANAGER V2.06E Operator: Adamek

Date	Time	Sample name	Weight [mg]	Standard name	Category	Protein factor	Peak Area [mV*s]	N Weight [mg]	Nitrogen [%]	Protein [%]
24.04.2012	14:20:55	3289	150,005		A 1,2	5,71	2,755E+02	5,569E-02	0,037	0,212
27.04.2012	09:31:05	3289	151,690		A 1,2	5,71	2,756E+02	5,570E-02	0,037	0,210
15.05.2012	11:02:32	3289	150,058		A 1,2	5,71	2,731E+02	5,506E-02	0,037	0,210
15.05.2012	11:06:20	3289	148,009		A 1,2	5,71	2,690E+02	5,402E-02	0,037	0,208
22.05.2012	10:30:41	3289	151,604		A 1,2	5,71	2,764E+02	5,592E-02	0,037	0,211

Average	0,037	0,210
Standard Deviation	0,00	0,00
RSD [%]	0,62	0,65

Calibration # : 46 (Cubic, With Zero)
Analysis Conditions for Method : Method_1
Sample Table : EDTA 3385

Temperatures:
Combustion Reactor 989 °C
Reduction Reactor 649 °C
Degassing Oven 299 °C

Times:
Sample Delay 5 s
Sample Stop 9 s
Run Time Auto

Flow Rates:
He I 195,0 sccm
He II 199,9 sccm
O_2 399,9 sccm

Figure 7:
Wheat starch with constant sample weight of approx. 150 mg.

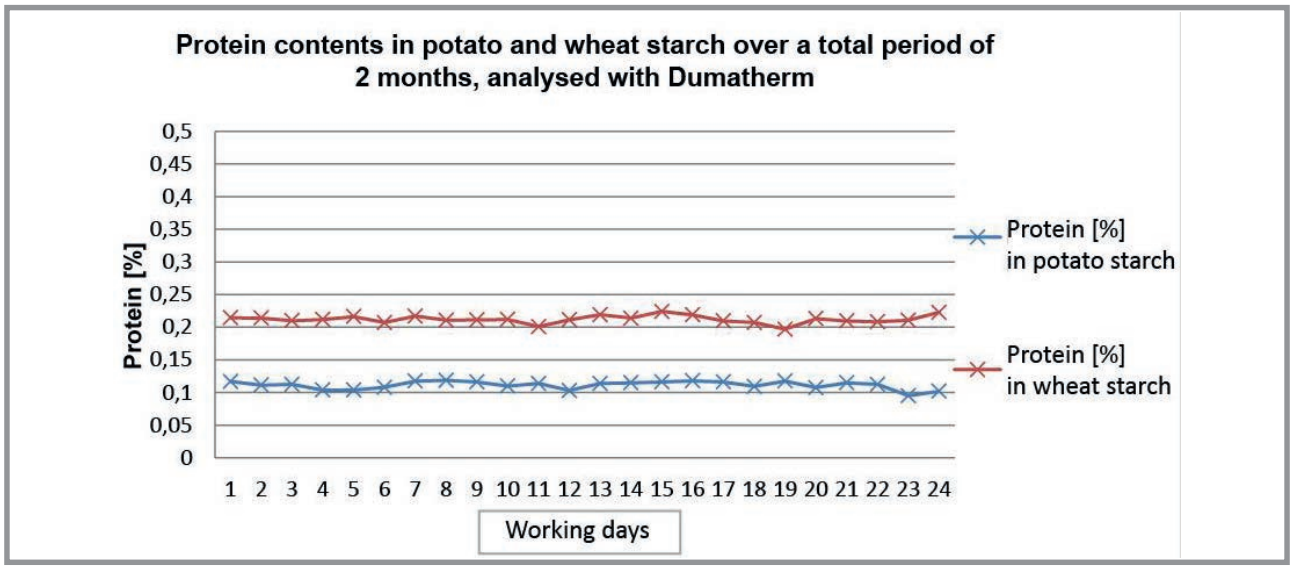

Figure 8: Long-term stability of the calibration over a total period of 2 months, measured on 24 different working days.

As a special feature, the potato starch was also measured with very different sample weights where excellent relative standard deviations of less than 0.6 % were obtained. This shows that sample weights of only 100 mg are necessary for the analyses. The peak areas obtained of approx. 200 mVs are very close to the absolute detection limit of 0.01 mg N.

2.5 Long-term stability of the calibration

The calibration created had very good stability. Using a potato starch and a wheat starch sample which were continuously analysed in a daily routine over almost 2 months for a total of 24 working days, it could be demonstrated that the newly developed calibration makes stable high-precision analysis with the DUMATHERM® possible in the range of less than 0.25 % protein. The results are shown in **Figure 8**.

2.6 Conclusion

Nitrogen / protein analysis according to the Dumas principle with the combustion apparatus available today is a credible alternative to the acid digestion method according to Kjeldahl. Despite only low sample weights, excellent reproducibility and repeatability are possible. The calibration strategy presented here enables reliable analysis of nitrogen content at the absolute detection limit of 0.01 mg N up to gluten samples with high protein content (60-80 % protein). As the examined starch had excellent homogeneity, the best analysis results were even possible with only 100 mg sample weight. This eliminates the usual problems of strong foaming and high chemical consumption in Kjeldahl digestion and a quantitative analysis can be obtained quickly and cleanly using combustion apparatus.

Therefore, it is not without reason that the combustion method is increasingly establishing itself as second reference method in addition to Kjeldahl.

Literature

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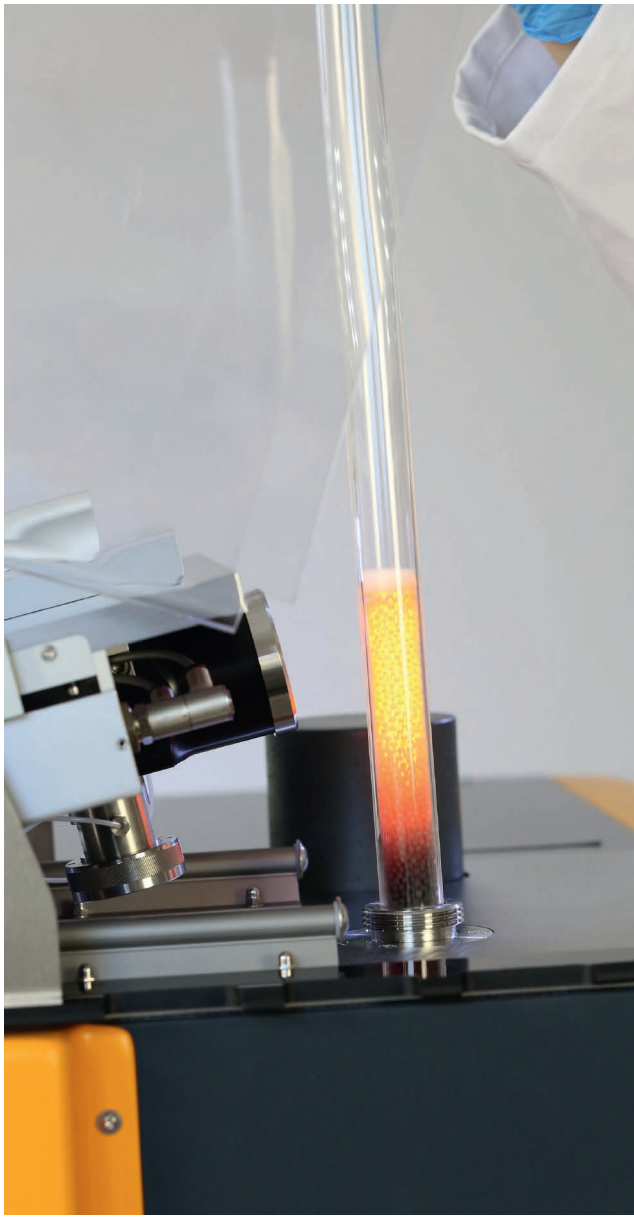

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