Gerhardt
Analytical Systems

Case study: Dumas nitrogen determination

Are macro sample weights at macro costs still viable today?

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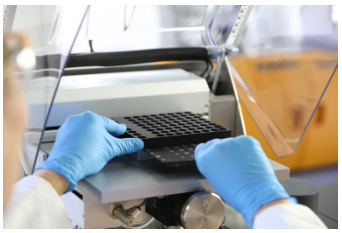

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Today, the question of speed and cost efficiency seems more relevant than ever in light of our need to conserve resources. The DUMATHERM® nitrogen analysis device, produced by C. Gerhardt GmbH & Co. KG, in Königswinter, Germany and operated according to the Dumas method, shows that these challenges need no longer be irreconcilable. Combustion catalysts without chrome constituents, non-toxic absorbents for water and carbon dioxide, as well as chemical-free solutions for the separation of carbon dioxide from the analytical gas stream, demonstrate this fact conclusively. If we are then able to use micro sample weights in place of gram weights on the basis of intelligent analytical technology, we can consequently view the combustion analysis in a new light, as it becomes a real alternative to both secondary methods and the Kjeldahl reference method.

This article aims to establish whether gram sample weights are still viable today, or whether intelligent detection technology paired with efficient equipment design render gram weights redundant. Flour and soybean are taken as sample matrices.

1. The general principle of combustion machines

For nitrogen/protein determination, samples are converted into their oxides through combustion at high temperatures with the presence of catalysts. The resulting nitric oxide (NO_2) is then transformed into elementary nitrogen using copper. The water and carbon dioxide by-products are separated completely before the remaining nitrogen is analysed with a thermal conductivity detector (see equation). The specific protein content is then calculated from the nitrogen content using the documented protein factor. The calculation is as follows: protein content = nitrogen content x protein factor.

$$(C_xH_yN_2)_{(s/l)} + O_2\ (g) \rightarrow x\ CO_2\ (g) + y\ H_2O\ (g) + z\ NO_{2\ (g)}$$

Analysis devices that function according to this principle comply with national and international

Nitrogen/protein analysis of flour using the DUMATHERM® nitrogen analyser with different sample weights (100-500mg) measured on various days.

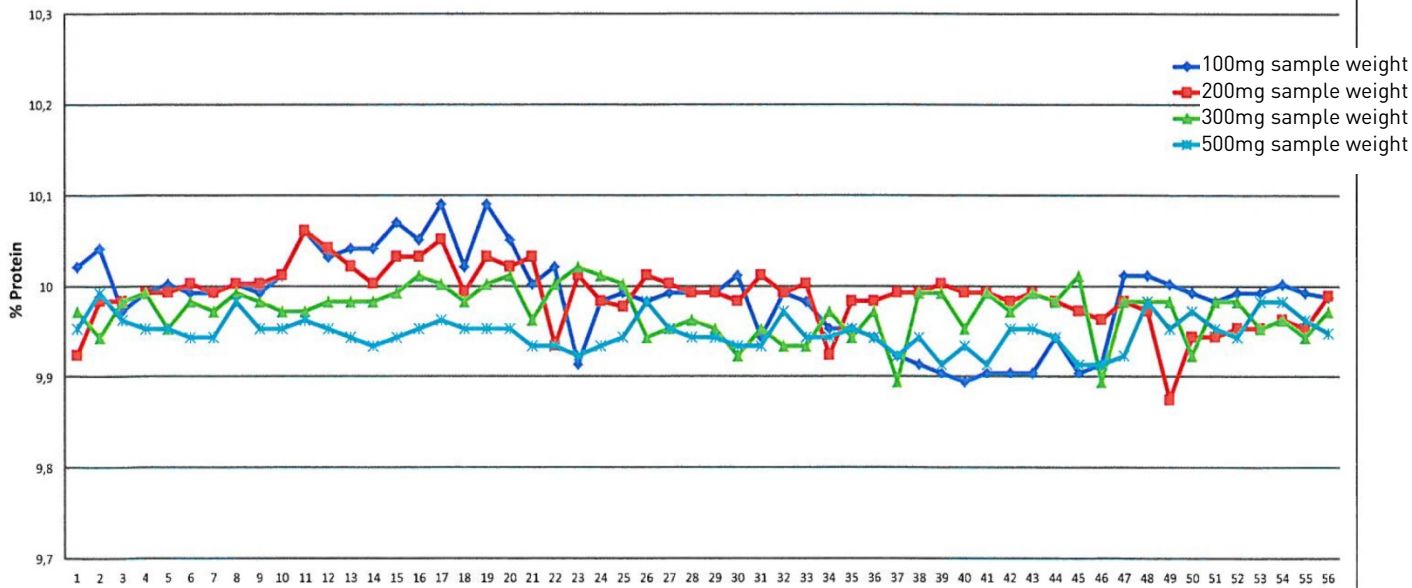

Fig. 1.: Protein values of flour (type 405), measured on 56 days using DUMATHERM. The day and the protein content are plotted here; the protein factor is 6.25.

standards and analysis regulations and fulfil the specified analytical quality requirements. Dumas devices are used across many fields of flour production and processing including, of course, in reference analyses for nitrogen/protein parameters, but also in direct production control using the Dumas reference method. And it is here that the speed of the Dumas method - which takes three to four minutes per analysis - proves particularly advantageous.

For Dumas devices, as for all analytical systems, the preparation of samples plays a decisive role. In many German standards, for example DIN EN ISO 166341, a sample weight of at least 100mg is required. Weight measurements with an analytical balance should also produce a reading accuracy of 0.0001g.

The following tests aim to show that conventional crop samples can measured using moderate sample weights - without difficulties and with excellent repeatability. This means the need for gram weighting can be conclusively discounted and the focus can instead be shifted to high throughput, speed and conservation of resources.

2. Repeatability in protein content for flour

If there is a repeated measurement of the same type of flour sample (flour type 405, without sample preparation) over a long period (56 measuring days over a total of 11 weeks, with over 200 measurements), in daily routine and in different sample weights (100, 200, 300 and 500mg), this renders the results illustrated here in Fig. 1 as average values of the four-tiered determinations.
The following conclusions can be derived from Fig. 1:

- The fluctuations in the measurement values are, as expected, larger for micro sample weights (100, 200 mg) than for macro sample weights (300, 500 mg).
- The average value of all these measurements at the different sample weights is almost the same in each case and, as such, there is no apparent matrix effect.

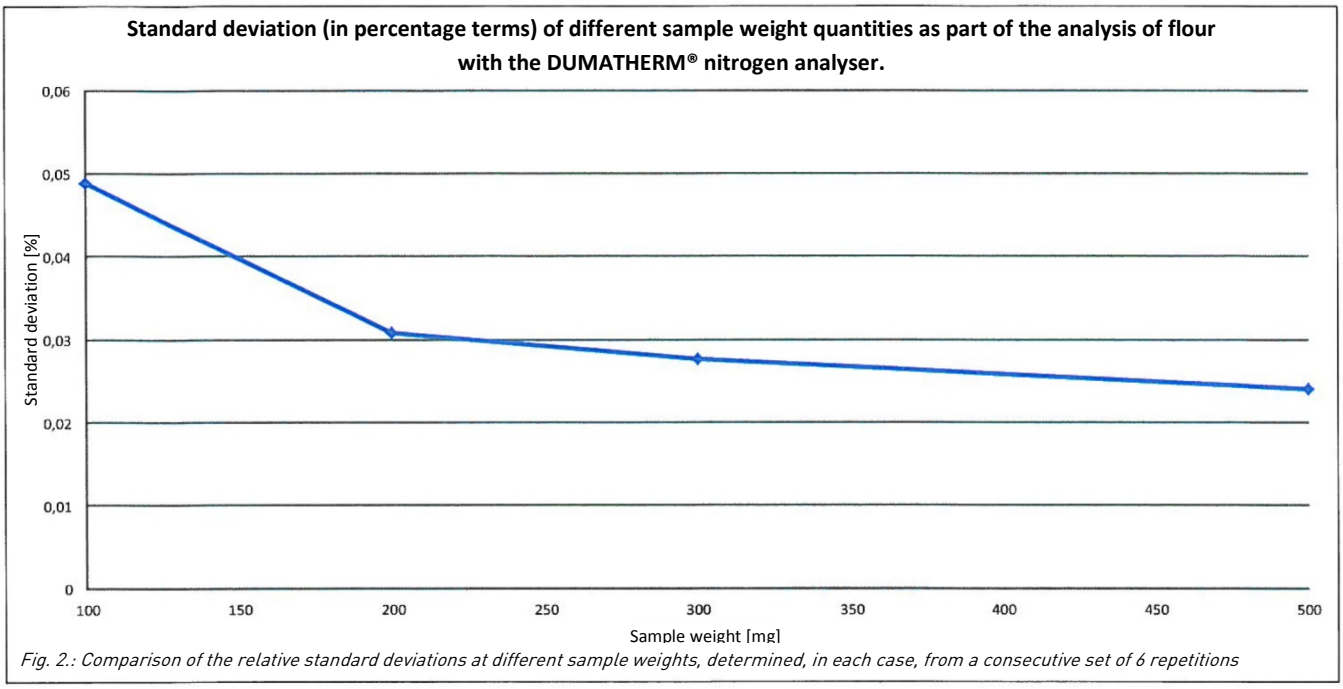

Fig. 2.: Comparison of the relative standard deviations at different sample weights, determined, in each case, from a consecutive set of 6 repetitions

If the standard deviation (in percentage terms) is plotted against the sample weight, this provides convincing evidence of the precision of modern analytics devices (Fig. 2).

Conclusion: from 200mg upwards, the relative standard deviation barely changes to any significant degree and the repetition of a consistently good standard deviation is guaranteed. For this reason, larger sample weights can be done away with - and not just for reasons of cost. There must also be a consideration of the interaction between the air in the laboratory and the sample matrix, in the case of open sample weight vessels.

The sample weight volume at 500mg can be assessed from a purely visual perspective in the following diagram (Fig. 3). A 1 cent coin is used for size-comparison purposes.

Fig. 3.: 200mg of flour compared to 500mg of flour and a 1 cent coin.

Combustion devices usually operate with an ash collection insert (Fig. 4) for the separation of unavoidable ash residue during combustion. These are gas-permeable ceramic frits which are installed in the combustion reactor directly under the autosampler.

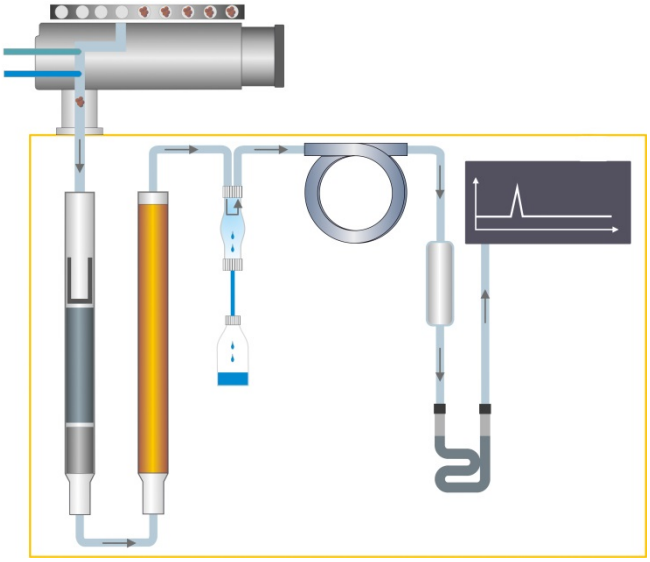

Fig. 4.: Analysis principle of a combustion device according to the Dumas principle with an Autosampler, combustion reactor with ash collection insert and a reduction reactor filled with copper and systems for water and carbon dioxide separation. Finally, a thermal conductivity detector and external PC for data evaluation

In order to avoid the higher costs associated with high sample weights and high ash levels, it is advantageous to have the lowest possible sample weights. The dispersed sample matrix that results during combustion with an open sample weight vessel also seems undesirable. All of this can be avoided using a sealed foil cover and, furthermore, the energy that is released from the burning of this foil can be used to reduce the combustion temperature. This means that modern combustion devices can be operated at a temperature of 950°C instead of 1,000°C.

3. Individual analyses

For additional precision, the results of the various measurements must be analysed again more carefully. If the flour is measured in series - on one day, as multiple determinations, and with a sample weight that remains the same -

Dumatherm Nitrogen / Protein Analyser

Serial Number : 159 Submitter :
Software Version: DUMATHERM MANAGER V4.11 Operator : Serviceman

Date	Time	Sample name	Weight [mg]	Protein factor	Nitrogen Peak Area [mV*s]	N Weidht [mg]	Nitrogen [%]	Protein [%]
04.09.2013	13:19:20	Mehl	200,781	5,70	1,333E+04	3,517	1,752	9,98
04.09.2013	13:23:42	Mehl	200,160	5,70	1,328E+04	3,505	1,751	9,98
04.09.2013	13:28:04	Mehl	200,539	5,70	1,332E+04	3,516	1,753	9,99
04.09.2013	13:32:21	Mehl	199,964	5,70	1,329E+04	3,507	1,754	10,00
04.09.2013	13:36:39	Mehl	200,368	5,70	1,332E+04	3,516	1,755	10,00
04.09.2013	13:40:59	Mehl	200,342	5,70	1,331E+04	3,512	1,753	9,99
04.09.2013	13:45:19	Mehl	200,599	5,70	1,333E+04	3,518	1,754	10,00

Average	1,753	9,99
Standard Deviation	0,001	0,01
RSD [%]	0,070	0,09

Calibration number and standard name : for N 3385 August 2013 (L-Q-Q) EDTA
Method : B 1,2
Series Name : Aug 13

Temperatures:		Flow Rates:		Times:	
Combustion Reactor	979 °C	He I	193,0 sccm	Sample Delay	7 s
Reduction Reactor	650 °C	He II	199,0 sccm	Sample Stop	11 s
Degassing Oven	299 °C	O_2	300,0 sccm	Run Time	Auto

Fig. 5.: Measurement result with 200mg of wheat flour, type 405

an excellent, absolute standard deviation of 0.01% is produced (Fig. 5).

3.1 Sample weight of 200mg of wheat flour, type 405

In the case of these multiple determinations, the standard deviation is smaller than 0.01% (Fig. 5). Sample weights of 200mg are clearly sufficient to achieve precise, and above all representative results with flour type 405.

3.2. Different sample weights: soybean example

In the case of feed that is highly inhomogeneous, the problem of bad repeatability at micro sample weights usually arises due to the sample itself. To solve this problem, one can either increase the sample weight or, as a better alternative, grind the sample so that it is finer.

Results of the first strategy: Increasing the sample weight

Table 1: Average values in protein determination using soybean (directly from the production line) at different sample weights. Average value of eight measurements.

Sample weight [mg]	Protein value [%]	Standard deviation [%]
200	45.91	0.65
300	46.56	0.32
400	46.44	0.25

While the average value remains almost unchanged, the standard deviations decrease as the sample weights increase, as was to be expected. The inhomogeneity of the sample is clearly recognisable in these values, however.

Results of the second strategy: Finer grinding of the sample

Fig. 6 shows the results of grinding down to a mesh size of 0.5mm (right). It is possible to see a direct improvement with the naked eye.

As a result, the standard deviation should improve with the finer grind. Table 2 shows the results.

Soybean sample taken directly from production

Soybean samples ground to a mesh size of 0.5mm with the Pulverisette 14 laboratory mill

Fig. 6.: Soybean samples prepared in different ways

Table 2: Comparison of different grinding fineness levels: Production grinding and 0.5mm grinding. Average value of eight measurements.

Grinding fineness	Protein value [%]	Standard deviation [%]
Production grinding, sample weight: 200mg	45.91	0.65
Grinding fineness: 0.5mm, sample weight: 200mg	46.45	0.14

A finer grind, as expected, reduces the relative standard deviation dramatically. As a result of the multiple determination process, the average values are almost the same throughout. The time and cost factor of the multiple determination process as measured against the finer grind must be weighed up.

A Kjeldahl determination, carried out as a comparison, with sample weights in grams, produced an average value of 46.42% protein. The comparison of methods thus produced identical values, without any concerns about deviating results.

For this reason, there are always several possibilities for the optimisation of measurement results for inhomogeneous products. To avoid a detailed discussion on the statistical aspect of the analytical results, this finer grade of grinding offers a pragmatic and fast solution.

4. Conclusion:

The DUMATHERM® is a highly-efficient, precise and fast analytic device for protein determination and represents, for most sample matrices, a genuine alternative to traditional processes such as Kjeldahl. Using the Dumas combustion method, the sample material is burned at high temperatures and the resulting combustion gases are analysed.

Thanks to its intelligent design, the DUMATHERM® features very few parts that are subject to wear, which reduces maintenance requirements to a minimum. The monitoring and operation of the device is performed entirely via PC using the DUMATHERM® Manager control software, and a result is available approximately three minutes after the reference method.

5. References

Cereals, pulses, milled cereal products, oilseeds, oilseed residues and animal feeding stuffs - Determination of total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content. - ISO/DIS 16634:2006; German version PrEN 16634:2006; DIN EN ISO 16634 - Draft

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VAPODEST® – fast distillation system for Kjeldahl nitrogen/protein determination and water steam distillation as sample preparation for further analysis.

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