The Reference Protein Determination Methods Kjeldahl and Dumas in Comparison -

Which method is the better choice for the analytical scope in my lab?

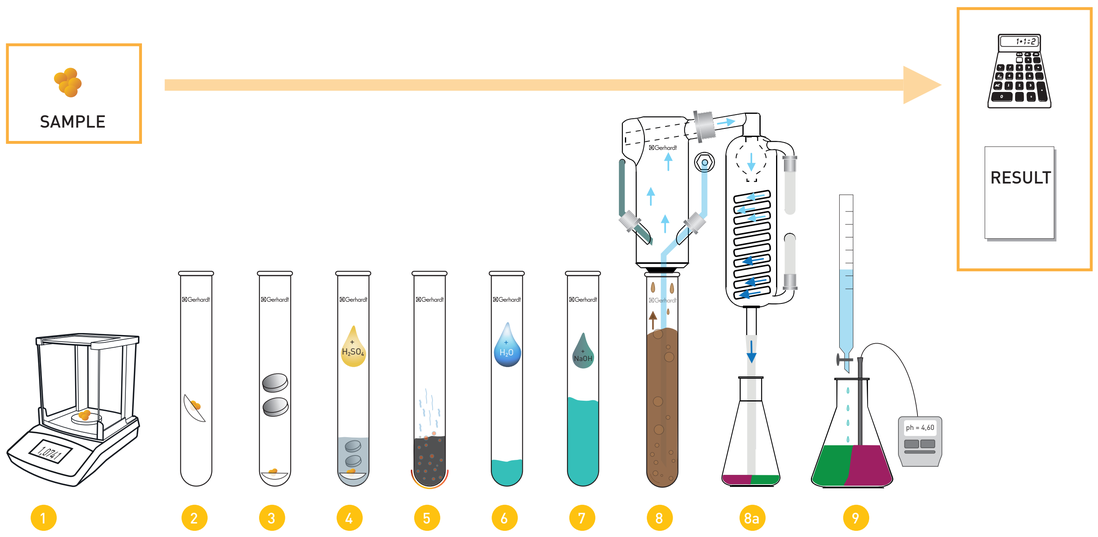
Dr. Werner Küppers, C. Gerhardt GmbH & Co. KG

Abstract

For the determination of nitrogen and protein in food, there are different established methods. In the methods discussed here the nitrogen content of a sample is analysed and then, the protein content is calculated using a protein factor which is specific for the sample type: As well as the classic Kjeldahl method, nowadays the faster Dumas method has become more and more accepted. This can be seen from the fact that the Dumas method is indicated as an additional reference method in various international standards. The author of this poster compares both methods, identifies decision criteria for and against these methods and illustrates the analytical performance by means of ring test results for different sample matrices.

Kjeldahl

$$C_nH_mN_x + H_2SO_4 \xrightarrow[\text{[catalyst]}]{400\ °C} n\ CO_2 + ½\ m\ H_2O + ½\ x\ (NH_4)_2SO_{4\ (solv)}$$



The Kjeldahl Method

The sample is weighed into nitrogen-free weighing paper (1) and is transferred into a digestion tube or flask together with the paper (2). Then salt is added which raises the boiling point and serves as a catalyst, for example KJELCAT Cu (3). Next step, sulphuric acid is added (4) and the sample is digested at boiling point for 60 to 180 minutes
[$C_nH_mN_x + H_2SO_4 \longrightarrow n\ CO_2 + ½\ m\ H_2O + ½\ x\ (NH_4)_2SO_{4\ (solv)}$] (5). The digestion solution is diluted with water to prevent strong reactions when adding sodium hydroxide solution in the next step in order to release the ammonia (6). With modern distillation units, e.g. VAPODEST®, the sodium hydroxide solution is dosed automatically [$NH_4^+ + OH^- \longrightarrow NH_3\uparrow + H_2O$] (7). Then, the ammonia is separated by steam distillation (8). The condensed ammonia-water-mixture is trapped in boric acid [$NH_3 + H_3BO_3 \longrightarrow NH_4^+ + H_2BO_3^-$] (8a). The quantitative content of nitrogen is determined by titration with sulphuric acid or hydrochloric acid, either by direct pH-measurement or by indirect measurement using a pH indicator
[$NH_4^+ + H_2BO_3^- + HCl \longrightarrow NH_4Cl + H_3BO_3$] (9).
The calculation of the nitrogen content in the sample is performed automatically with modern distillation units, e.g. VAPODEST® 500, by connected software. The protein content of the sample is calculated based on the nitrogen content.

Technical realisation by C. Gerhardt

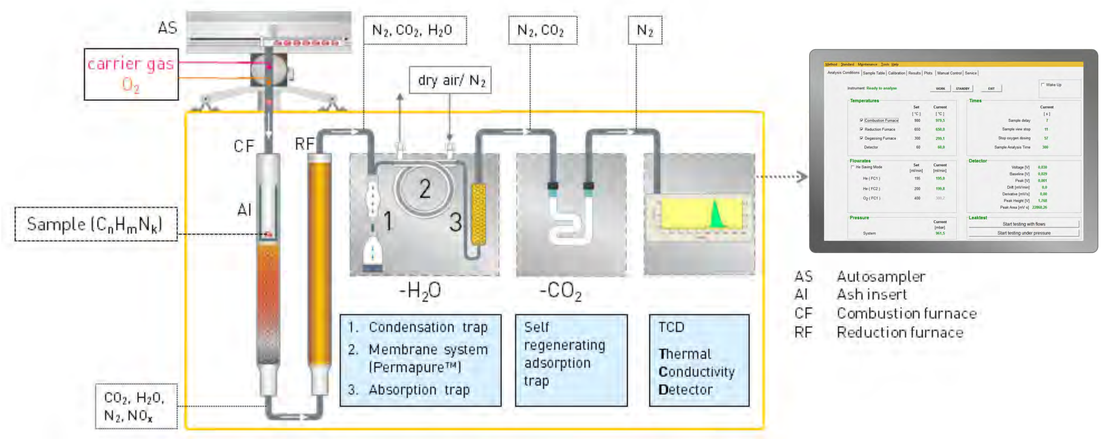
Digestion Unit
KJELDATHERM®

Scrubber Unit
VACUSOG

Distillation System
VAPODEST® 500

Dumas

$$C_nH_mN_x + a\ O_2 \xrightarrow[\text{[catalyst]}]{1030\ °C} n\ CO_2 + ½\ m\ H_2O + x\ \text{N-Oxides} \xrightarrow[\text{[Cu]}]{800\ °C} ½\ x\ N_2$$



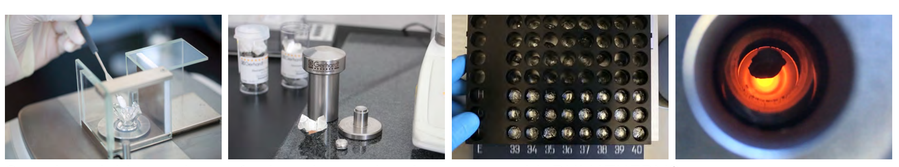
The Modern Dumas Procedure

In a carrier gas flow (helium), the airtight packed samples are conveyed to the combustion process performed with pure oxygen. Catalysts accelerate the combustion and eliminate the problem of CO formation.
The nitrogen oxides are reduced to elemental N over highly active copper powder. Water and carbon dioxide is separated using appropriate absorbents, for example with a regenerative molecular sieve for CO_2 and gas dryers for the water.
The % N content of the samples is calculated by an evaluation software based on the quantitative determination of the nitrogen content with a thermal conductivity detector. The detector is calibrated using a reference substance.

The Classic Dumas Procedure

The sample is mixed with copper oxide and is heated in a carrier gas flow of carbon dioxide. The formed gas is led over a layer of CuO and is hereby oxidised to the desired products CO_2, water and nitrogen oxides. The nitrogen oxides are reduced to elemental nitrogen with help of copper wire. The nitrogen is led through a potassium hydroxide solution and is trapped in a graduated cylinder. The nitrogen content can be quantitatively metered from the displaced volume of the liquid. The side products carbon dioxide (CO_2) and water (H_2O) are either precipitated as carbonate in the potassium hydroxide solution or remain in the solution. The % N proportion of the sample can be back calculated from the determined nitrogen volume, based on the initial sample weight.

The modern technical solution from C. Gerhardt



Sample weighing and transfer into the combustion reactor with DUMATHERM®

Positive Points for the Kjeldahl method

- Still THE universal reference method for all sample matrices
- Highest flexibility in sample size
- Wide range of configurations possible to suit budget
- Perfect for low sample throughput
- Conform with international standards
- Ideal with constantly changing applications

Points for the Dumas Analysis

- Rapid analysis within 3 minutes
- Virtual chemical-free process, exhaust equipment not required
- Low usage of consumables - low cost of analysis
- Conformity with international standards
- Easy conditioning of the system in the routine, with easy maintenance
- Low gas usage - up to 32,000 analyses with one oxygen bottle, for example

Comparison of both methods using ring test samples which have been analysed with both methods by different laboratories. The following table shows the results from both methods and compares the mean values [%N].

Sample	Ring Test Organisation	[%] Nitrogen Dumas		[%] Nitrogen Kjeldahl		Δ Dumas-Kjeldahl [%] N	Ring Test Mean Value [%] N Kjeldahl	Upper Limit Kjeldahl	Lower Limit Kjeldahl
Gluten meal	GAFTA	mean value	3.592	mean value	3.529	0.064	3.560	3.766	3.354
		sd	0.015	sd	0.007				
Basmatic rice	GAFTA	mean value	1.380	mean value	1.350	0.030	1.363	1.321	1.404
		sd	0.008	sd	0.004				
Soya meal	GAFTA	mean value	7.699	mean value	7.691	0.008	7.602	7.702	7.503
		sd	0.020	sd	0.096				
Barley	GAFTA	mean value	1.255	mean value	1.230	0.025	1.210	1.249	1.170
		sd	0.010	sd	0.026				
Boiled sausage	LVU	mean value	2.318	mean value	2.295	0.023	2.259	2.381	2.126
		sd	0.007	sd	0.012				
Maize snack	FAPAS	mean value	1.647	mean value	1.652	-0.005	1.66	1.722	1.598
		sd	0.017	sd	0.016				
Meat	AOAC	mean value	1.823	mean value	1.794	0.029	1.801	1.834	1.768
		sd	0.092	sd	0.021				
Porridge	FAPAS	mean value	1.916	mean value	1.860	0.056	1.820	1.960	1.690
		sd	0.008	sd	0.010				
Infant formula	FAPAS	mean value	1.719	mean value	1.675	0.044	1.670	1.800	1.550
		sd	0.007	sd	0.006				
Yoghurt	MUVA	mean value	0.771	mean value	0.770	0.001	0.771	0.781	0.761
		sd	0.002	sd	0.003				
UHT milk	MUVA	mean value	0.551	mean value	0.549	0.002	0.553	0.559	0.547
		sd	0.003	sd	0.002				
Whey protein powder	MUVA	mean value	4.889	mean value	4.871	0.018	4.830	4.848	4.814
		sd	0.005	sd	0.007				
Cream	MUVA	mean value	0.381	mean value	0.370	0.011	0.378	0.383	0.373
		sd	0.003	sd	0.001				

Remark: sd = standard deviation [%]. All mean values and standard deviations [sd] have been determined by at least 6 analyses per sample at the same unit.
The column Δ Dumas-Kjeldahl [%] N shows the difference between the mean value Dumas and the mean value Kjeldahl.

Conclusion from the Analysis Results:

In nearly all cases, the standard deviation (sd) of the Dumas method is the same or better than the standard deviation of the Kjeldahl method, despite the smaller sample weight (300 mg instead of 1 g at Kjeldahl). In each case, the standard deviation is within the allowed tolerance of the corresponding Dumas standards, such as DIN ISO 14891 for milk and dairy products or AOAC 992.15 for meat products. Lying within the allowed variations of the Kjeldahl standards, all Dumas values would comply with the quality requirements of the Kjeldahl standards, too. This means, the precision of the Dumas values is high enough and comparable to the Kjeldahl values.

The measured data show that there is no basis for a discussion about higher Dumas values in general.

From the multiplicity of the compared data the question arises, whether such a discussion is still up to date.

Typically, differences occur, when strongly fertilized plant products are analysed with both methods, for example soya meal. In this case, the inorganic nitrogen comes into effect, which is not detected by the classic Kjeldahl analysis unless Devarda alloy is added. But still in this case, no significant deviation of values can be seen from the quoted ring test results, except with the gluten meal from GAFTA.

Conclusion:
Since the Dumas method has been established more and more as a reference method for different applications (also refer to the wide method list below), an increasing popularity of this method can be surely expected because of its distinctive advantages.

The Dumas method in international standards and methods (extract)

Meat
- AOAC 992.15
- § 64 LFGB 06.00-20

Grain
- AOAC 992.23
- AACC 46-30
- ICC Standard No. 167
- GB/T 31578-2015
- NYT 2007-2011

Milk and dairy products
- AOAC 992.15
- DIN EN ISO 14891 (IDF 185)
- § 64 LFGB 01.00-60
- § 64 LFGB 02.00-24
- § 64 LFGB 03.00-27
- GB 5009.5-2016

Food
- DIN ISO EN 16634-1 /GB/T 24318-2009
- ISO TS 16634-2
- AOCS Ba 4f-00
- § 64 LFGB, methods 17.00-18; 18.00-18; 22.00-2; 48.01-26
- SN/T 2115-2008

Gerhardt Analytical Systems