Kjeldahl – still going strong

Some 125 years ago the Danish chemist Johan Kjeldahl published a method that basically is still in use in the food and agri industry:

When a protein containing sample is boiled in sulphuric acid, nitrogen is transformed into ammonium sulphate. Salt is added to give a higher boiling point and thereby quicker analysis. Also the reaction is speeded up by adding a catalyst. Today copper sulphate has replaced mercury oxide as the most commonly used catalyst. The ammonium sulphate is determined by adding an excess of alkali to the diluted digest. The ammonia thus liberated is distilled into a receiver solution (today boric acid, in older applications sulphuric acid) and titrated. The percentage of protein is found by multiplying the percentage of nitrogen by standard factors depending on the sample type. These factors were derived on basis of the amino acid composition of samples. The factor 6.25 is the most generally used one, whilst e.g. 5.7 is applied for wheat and 6.38 for dairy products.

FOSS pioneering the Kjeldahl method

In 1970 FOSS introduced the Tecator™ block digestion to replace Bunsen burners and 800 ml Kjeldahl tubes for digestion. In 1974 direct steam distillation was introduced with the FOSS Tecator Kjeltec™ systems. These two major improvements led to a decreased use of chemicals and an improved efficiency of the digestion. No sample transfer was necessary any longer as the sample is digested and distilled in the same tube. The risk for ammonia losses was eliminated by distillation in a closed system and distillation into boric acid receiver solution reduced the distilling times.

In 2009 the latest generation of modern Kjeltec analysers is introduced (see page 4).

Modern Standards for Crude Protein

At the end of the 1980's/ beginning of the 1990's there were thousands and thousands of laboratories using block digestion and steam distillation equipment for determining the Kjeldahl protein, but the existing standards were not reflecting the instrumental improvements for the Kjeldahl method. They were i.e. still applying mercury catalysts, using large amounts of sulfuric acid and long digestion times, making "normal" titration into sulfuric acid and using back titration with sodium hydroxide, which contributed to the breakthrough of the Dumas combustion method and NIR methods. So there was a need for updated standards.

At the end of the 1990's FOSS developed a new standard method in cooperation with the AOAC using block digestion with copper sulfate catalysts, a minimum of sulfuric acid and a digestion time of 1 hour, steam distillation into boric acid receiver solution and simultaneous titration with photometric endpoint determination, reducing the distillation/titration time to some 3-4 minutes[1, 2, 3, 4]. This standard was later adopted by ISO and CEN[6] as EN ISO 5983-2 establishing a global standard for crude protein determinations[5].

The standard was validated on a broad range of samples, covering a wide concentration range (see fig. 2) with participating laboratories using FOSS Tecator Kjeltec equipment. The sample types covered represented animal feed, forages/plant material, cereal grain, oil seeds, pet food, protein concentrates, milk powder and aquafeed.

The results show an excellent repeatability and reproducibility (0.4 – 2.4 %). Recoveries of nitrogen from tryptophan were 98.8 % and from acetanilide 100.1 %. Blind duplicates lined up nicely, showing that the method is sturdy and reliable. Figure 3 shows the precision values in terms of standard deviations for the repeatability, sdr, and reproducibility, sdR, which can be used for calculating the measurement uncertainty values[8]. The precision values are dependent on concentration. For some samples, there was a difference between sdr and sdR, e.g. for fish meal. Otherwise, the figure shows that there is no significant difference between repeatability values and reproducibility values, which means all labs can produce correct results (as all did use Kjeltec instruments).

The method EN ISO 20483:2008 Cereals and pulses – Determination of the nitrogen content and calculation of the crude protein content – Kjeldahl method[7] is using block digestion with a Copper sulfate/ Titanium oxide catalyst, steam distillation into boric acid, automatic titration with photometric (or pH) end point determination. This new method differs from EN ISO 5983-2/AOAC 2001.11 mainly with respect to the digestion time (2 h instead

Fig 1: This Kjeltec™ model 1001 from 1974

Facts about the samples

- Validation samples:
  - Sample 1: protein block
  - Sample 2: swine pellets
  - Sample 3: corn silage
  - Sample 4: grass hay
  - Sample 5: fish meal
  - Sample 6: dog food
  - Sample 7: chinchilla feed
  - Sample 8: albumin
  - Sample 9: bird seed
  - Sample 10: meat and bone meal
  - Sample 11: milk replacer
  - Sample 12: soybeans
  - Sample 13: sunflower seed
  - Sample 14: legume hay
  - Sample 15: fish feed, small floating pellets
  - Sample 16: fish feed, large floating pellets
  - Sample 17: shrimp feed, crumble
  - Sample 18: shrimp feed, large sinking pellets
  - Sample 19: shrimp feed, small sinking pellets
  - Sample 20: larvae feed, flake
  - Sample 21: wheat grain
- Validated range: 0.3 – 70 % protein
- Validated by 24-26 international laboratories
- Photometric end point determination
- Developed on basis of FOSS Kjeltec™ equipment
- Reference method also for Dumas
- Excellent repeatability and reproducibility

Fig. 2: Performance of the EN ISO 5983-2 / AOAC 200.11 standard

of 1 h), amount of acid (20 ml vs 12 ml) and catalyst (Copper sulfate/Titanium oxide vs Copper sulfate only) used.

The precision data of this standard (fig.3, right) show a significant difference between repeatability and reproducibility values. This may be due to the fact that FOSS Kjeltec equipment has not exclusively been used in this study.

The promulgation of Titanium oxide as catalyst stems from investigations made when trying to replace Mercury oxide. On nitrogen test substances better recoveries were found compared to Copper sulfate only.

Another way for nitrogen/ protein determinations is the Dumas combustion method as described in e.g. EN ISO 16634[9, 10]. It is using the same factors as the Kjeldahl method for the calculation of the protein content, but its application has to be verified against the Kjeldahl method.

The Dumas combustion method determines the total nitrogen including inorganic fractions and has a tendency to give higher protein values than the Kjeldahl method. Kjeldahl does not recover inorganic fractions like nitrite and nitrate and is not recovering all organic nitrogen. For example, are heterocyclic compounds only partially recovered? On the basis of tens of thousands of data from our grain NIR calibrations, we know that the Dumas values will be some 0.1 – 0.2 higher in protein. This is usually negligible, but has to be taken into account.

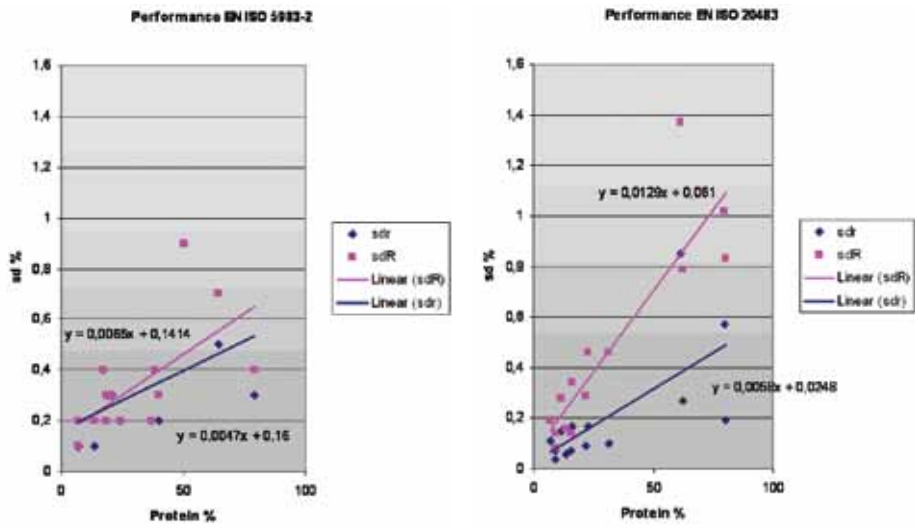

Fig. 3: Standard deviations of repeatability and reproducibility (in % protein) for the EN ISO 5983-2/ AOAC 2001.11 (left) and EN ISO 20483 (right) standards

Table 1 shows the nitrate contents of certified reference material from Wepal (Holland). If we take e.g. lettuce with a nitrate content of 33 200 mg/kg nitrate as an example, then would this correspond to 7.5 g N/kg or 0.75 × 6.25 = 4.7 % Protein, indicating the kind of errors that can be produced.

This kind of difference may even lead to trade conflicts. If, let's say, the Argentine supplier of soymeal claims a protein content of 47.2 %, determined on basis of the Dumas method and the Malaysian importer of soymeal claims a protein content of 44.9 %, determined on basis of the Kjeldahl method, this may result in a conflict.

This is also the reason why the European Commission has recently confirmed the Kjeldahl method as the community method for official controls (Commission

CRM	Nitrate (g/kg d.m.)
French beans	8.9
Summer barley	0.1
Lettuce	33.2
Cucumber	7.2
Yam	4.9
Cabbage	7.1
Spinach	27.2

Table 1: Nitrate contents (% d.m.) in certified reference materials

Method	% CP
ICC 105/2 (Kjeldahl, Cu)	12.46
EN ISO 5983-2 (Kjeldahl, Cu)	12.45
ISO 20483 (Kjeldahl, Cu/Ti)	12.39
ISO 16634 (Dumas)	12.55

Table 2: Average protein content by different methods (average sd = 0.125)

Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed).

Which method to select?

Table 2 shows the average protein content obtained by different reference methods in the 2008/2009 interlaboratory study of the European Grain Networks. In this study, some 20 master laboratories analysed seven wheat and four barley samples. The average standard deviation of reproducibility of these methods was 0.125%.

Table 3 shows protein results obtained by different methods in the AAFCO proficiency testing scheme for 2008. The AAFCO is the Association of American Feed Control Officials. Some 200 labs are participating in their proficiency testing scheme. Different Kjeldahl methods (EN ISO 5983-2/ AOAC 2001.11 = Cu catalyst; EN ISO 20483 = Cu/TiO_2 catalyst) have been compared with the Dumas combustion method.

As the results in table 2 and 3 show, the differences between the methods, will in most cases, be insignificant. For grain and mixed feed samples there is no indication that the EN ISO 5983-2 / AOAC 2001.11 method using copper sulfate as catalyst only will generate lower results than the method using a mixture of copper sulfate and titanium dioxide. On the other hand, the Dumas method may lead to insignificantly higher values.

The choice of method for the determination of crude protein will therefore be governed by economical and practical reasons. The global standard EN ISO 5983-2 / AOAC 2001.11 has the advantage of broader applicability, shorter analysis times and lower reagent costs versus the EN ISO 20483 method and a better status as reference method versus the Dumas method.

It allows accreditation with full traceability[5] and easy estimation of the method uncertainty[8] for a wide range of samples.

	Cu		Cu/TiO_2		Dumas	
Sample	% CP	sd %	% CP	sd %	% CP	sd %
AAFCO 0826	18.2	0.35	18.2	0.29	18.4	0.32
AAFCO 0827	12.9	0.36	12.8	0.46	13.0	0.46
AAFCO 0828	40.8	0.77	40.0	0.68	41.0	0.43
AAFCO 0829	23.6	0.42	23.0	0.58	23.5	0.33
AAFCO 0830	18.2	0.27	18.2	0.24	18.5	0.32
AAFCO 0831	27.5	0.40	27.3	0.64	27.8	0.46
Average	23.5	0.43	23.2	0.48	23.7	0.39

Table 3: Protein content by different methods (AAFCO PTS 2008). % Crude protein and standard deviations for different methods

References:

1. Official Methods of Analysis, AOAC International, Gaithersburg, USA, Method 2001.11: Determination of crude protein in animal feed, forage (plant tissues), grain, and oil seeds using block digestion with a copper catalyst and steam distillation into boric acid

2. Nancy J. Thiex and Harold Manson, New AOAC Standard for Crude Protein Determinations in Animal Feed, Grain, and Oilseed using Tecator Kjeltec equipment, In Focus, vol 26, no 2, 2002, p 10-12

3. Thiex et al.: AOAC COLLABORATIVE STUDY REPORT – Crude Protein in Animal Feed, Forage, Grain, and Oilseed - Block Digestion Using Copper Catalyst, Steam Distillation into Boric Acid, Journal of AOAC International vol 85, no 2, 2002, 309-317

4. FOSS Application Note AN 3001

5. Jürgen Möller, Traceability in Modern Kjeldahl Analysis, In Focus, vol 29, No 1, 2005, p 4-5

6. EN ISO 5983-2:2005 Animal feeds – Determination of nitrogen content and calculation of crude protein content – Part 2: Block digestion/ steam distillation method

7. EN ISO 20483:2006 Cereals and pulses – Determination of the nitrogen content and calculation of the crude protein content – Kjeldahl method

8. Cheng Shuwei, Gu Jinling and Pang Jing, Quantifying the measurement uncertainty for protein determinations in soybeans using the Kjeltec 2300, In Focus, vol 32, no 1, 2008, p 24-27

9. ISO 16634-1:2008 Food products – Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content – Part 1: Oilseeds and animal feeding stuffs

10. ISO/PRF TS 16634 Food products – Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content – Part 2: Cereals, pulses and milled cereal products (Technical Specification under development)

■

by Jürgen Möller, jmr@foss.dk