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Dumas or Kjeldahl for reference analysis?

Comparison and considerations for Nitrogen/Protein analysis of food and feed

By: Dr. Jürgen Müller

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ANALYTICS BEYOND MEASURE

The Dumas method

The Dumas combustion method is an absolute method for the determination of the total Nitrogen content in a usually organic matrix. The sample is combusted at high temperature in an oxygen atmosphere. Via subsequent oxidation and reduction tubes, nitrogen is quantitatively converted to N_2. Other volatile combustion products are either trapped or separated. A Thermal Conductivity Detector measures Nitrogen gas. Results are given as % or mg Nitrogen, which may be converted into protein by using conversion factors (table 1).

Table 1 – Nitrogen to Protein conversion factors (examples from ISO 16634-1:2008)

Commodity	Conversion factor
Barley	5,88
Coconut meal	5,30
Oats	5,50
Rice	5,95
Rye	5,83
Sun flower (seed, meal)	5,30
Soy bean (seeds, flour or products)	5,71
Triticale	5,78
Wheat (whole meal, flour or bulgur)	5,83
Wheat (bran)	5,26

According to ISO/TS 16634-2:2009 the generally agreed conversion factor for the product analysed, is equal to 5,7 for wheat, rye and their milled products and 6,25 for all other products falling within the scope of this ISO standard.

During the 1990's the Dumas method gained recognition versus the traditional Kjeldahl method that was the dominating method for Crude Protein analysis for more than 100 years.

The Dumas method has the advantage of being easy to use and automated. It is also considerably faster than the Kjeldahl method, taking a few minutes per measurement, as compared to an hour or more for Kjeldahl. It also does not make use of toxic or harmful chemicals or catalysts. The Kjeldahl method uses concentrated sulfuric acid and a catalyst for digestion of samples. When the use of mercury and cadmium in the laboratory was banned in most countries during the 1990's, many laboratories evaluated the Dumas method as an alternative and numerous comparative studies have been made. One result of this recognition has been a number of international standards (table 2). Also grain inspection services in the US, Canada and Australia recognised the Dumas method.

As the Dumas method determines total Nitrogen including inorganic fractions like nitrite and nitrate and the Kjeldahl method only organic nitrogen and ammonia, differences in results occurred in the comparative studies. The Kjeldahl method does not recover all organic nitrogen and has problems especially with the recovery of heterocyclic N-compounds like e.g. nicotinic acid. This has not been seen as a problem, as in crude protein determinations the main issue was the conversion of alpha-amino nitrogen from amino acids into ammonia. Historically, the nitrogen to protein conversion factors for the traditional Kjeldahl method, have been established based on the amino acid pattern of the sample. For feed and food samples with varying composition, a general factor of 6,25 has been agreed on. When using the same conversion factors for techniques with different nitrogen recoveries, differences in results may occur.

Table 2 – Standards for total nitrogen and protein measurement in food and feed using the Dumas method

Method reference	Title (Matrix)
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 stuff
ISO/TS 16634-2:2009	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
ISO 14891:2008 (IDF 185:2008)	Milk and milk products — Determination of nitrogen content — Routine method using combustion according to the Dumas principle
AACC Method 46.30	Crude Protein — Combustion Method (Animal feeds, cereals and oil seeds)
ICC Standard No. 167	Determination of crude protein in grain and grain products for food and feed by the Dumas combustion principle
AOAC 990.03	Protein (Crude) in Animal Feed — Combustion Method
AOAC 992.23	Crude Protein in Cereal Grains and Oil Seeds
AOAC 997.09	Nitrogen in Beer, Wort, and Brewing Grains — Protein (Total) by Calculation — Combustion Method
AOCS Ba 4e-93	Generic Combustion Method for Determination of Crude Protein (Oilseed byproducts)
AOCS Ba 4f-00	Combustion Method for Determination of Crude Protein in Soybean Meal
OIV-MA-AS323-02A	Quantification of total nitrogen according to the Dumas method in Musts and Wines (Type II method)

AOAC = AOAC International, Washington DC/USA , AOCS = American Oil Chemists' Society, Champaign IL/USA, AACC = American Association of Cereal Chemists, St. Paul MN/USA, ISO = International Organization for Standardization, Geneva/Switzerland, IDF = International Dairy Federation, Brussels/Belgium, ICC = International Association for Cereal Science and Technology, Vienna/Austria, OIV = International Organisation of Vine and Wine, Paris/France

Dumas and Kjeldahl methods will lead to different results, depending on the non-protein-nitrogen content of the analysed sample and to what degree they are recovered by respective methods. For example, in a lettuce sample with a nitrate content of 33 000 mg/kg dry matter, this will correspond to 0,75% Nitrogen or 4,7% Crude protein (factor 6,25).

The AAFCO (American Association of Feed Control Officials) PTS is one of the most comprehensive proficiency testing schemes with some 300 participating labs and more than 100 reported methods. Table 3 shows the results for some feed samples. For Kjeldahl the values for the reference method AOAC 2001.11 have been selected. As can be seen, the standard deviations of reported results (in parenthesis) are comparable, but the Dumas method results in higher values.

Table 3: Comparison of crude protein values obtained for AAFCO proficiency testing samples

Sample	Type	Kjeldahl	Dumas
AAFCO 200921	Chicken	17,29 (0,15)	17,64 (0,33)
AAFCO 200922	Pig starter	23,94 (0,33)	24,51 (0,39)
AAFCO 200923	Chow	12,3 (0,52)	12,51 (0,65)

During five harvest years (2000 – 2004) the Max Rubner Institute in Detmold, Germany, performed a comprehensive study with more than 800 wheat samples comparing the crude protein results between Kjeldahl and Dumas methods. They found that some 2% of "Dumas protein" was not determined by the Kjeldahl method and presented the following relationship between Dumas and Kjeldahl protein values:

Kjeldahl = 0,959*Dumas + 0,258

As the difference between the methods not only depends on growing year and cultivar but also on growing conditions (i.e. rain, fertilisation) this formula could not generally be used for converting results.

Numerous other comparative studies have been reported. Simonne et al. (1) come to the conclusion that the Dumas method may replace the Kjeldahl method for the determination of Crude Protein in selected food groups when appropriate coefficients are used. They suggested correction factors of 1,01 for dairy, 1,00 for oilseeds, 0,99 for feed, 0,98 for infant formulas, 0,95 for cereals, 0,94 for meats, 0,89 for vegetables, 0,80 for fish and 0,73 for fruits when calculating Crude Protein using Dumas method results.

Others think that the overestimation is difficult to handle and to avoid trade conflicts the European Commission confirmed the Kjeldahl method as the community method for official controls (Commission Regulation (EC) No 152/2009).

With regard to selectivity, interferences, and susceptibility to adulteration it has to be kept in mind that the Dumas method quantitatively recovers all forms of nitrogen, organic and inorganic, during analysis and is specific for total nitrogen, it lacks any degree of selectivity for protein. Therefore, the method is susceptible to adulteration by all organic and inorganic compounds that contain nitrogen.

Just like the Kjeldahl method it does not give a measure of true protein, as it registers non-protein nitrogen, and different correction factors are needed for different proteins because they have different amino acid sequences (2). In fact, these correction algorithms are barely used in routine labs. In most cases the same factors are used to convert Dumas and Kjeldahl values into crude protein.

To avoid conflicts and misunderstandings in trade situations it is therefore of importance to clearly state the method to be used for crude protein determinations. In official situations, where Kjeldahl still is the most recognised method, Dumas can be used if differences for the type of samples analysed are negligible. It is also important to be aware of these differences when establishing and validating NIR calibrations.

Summary

For the estimation of the crude protein content in food and feedstuffs there is a clear trend towards the Dumas combustion method, as it offers shorter analysis times, ease of operation and improved safety compared to the Kjeldahl method. Both methods have similar precision but Kjeldahl is still the most recognised and can be highly effective, when run in batches. Both methods are susceptible to adulterations.

References:

(1) A H Simonne, E H Simonne, R R Eitenmiller, H A Mills and C P Cresman: *Could the Dumas Method Replace the Kjeldahl Digestion for Nitrogen and Crude Protein Determinations in Foods?* Journal of the Science of Food and Agriculture, Volume 73, Issue 1, pages 39–45, January 1997

(2) Jeffrey C. Moore, Jonathan W. DeVries, Markus Lipp, James C. Griffiths and Darrell R. Abernethy: *Total Protein Methods and Their Potential Utility to Reduce the Risk of Food Protein Adulteration*, Comprehensive Reviews in Food Science and Food Safety, Volume 9, Issue 4, pages 330–357, July 2010

FOSS

FOSS
Foss Allé 1
DK-3400 Hilleroed
Denmark

Tel.: +45 7010 3370
Fax: +45 7010 3371

info@foss.dk
www.fossanalytics.com