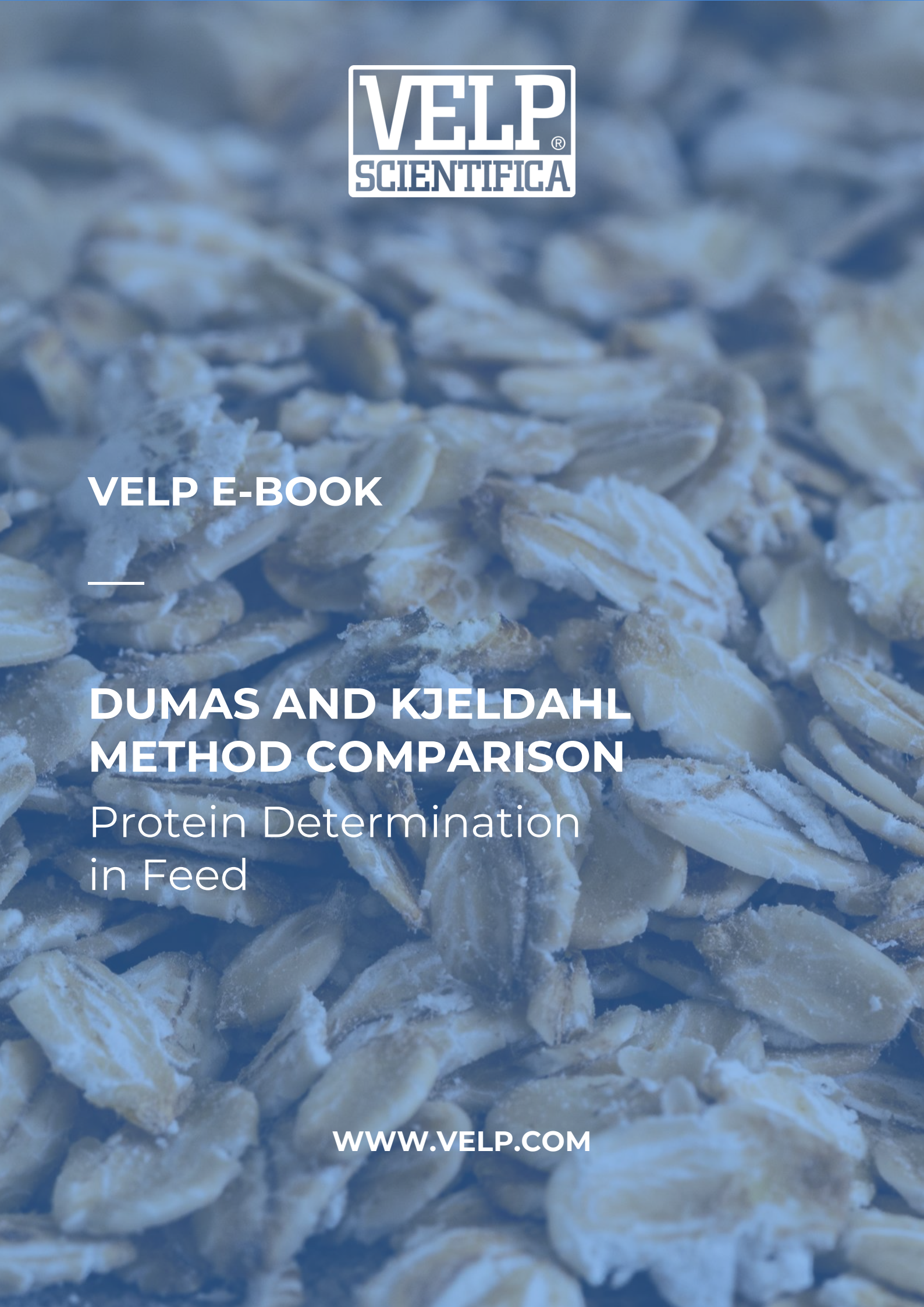

VELP E-BOOK

DUMAS AND KJELDAHL METHOD COMPARISON

Protein Determination in Feed

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PROTEIN CONTENT: TWO PRIMARY METHODS

As stated by international labeling regulations in the Food & Feed industry as well as research facilities, the **analysis of the total protein content** is key in relation to quality control and protein declaration.

For the **determination of the protein content** of food and feedstuff, **two distinct primary methods** have been widely accepted within laboratory operations. These are **Kjeldahl's wet chemical method** and **Dumas' high-temperature combustion method**. Near-infrared spectroscopy (NIRS) can be employed as a secondary means of analysis, but this process necessitates a primary method for calibration.

For over a century, the **Kjeldahl method** was the most frequently applied method and the industry standard for the determination of the **total protein content of food products** by measuring the total organic Nitrogen content.

As an analysis involving wet chemicals, it requires excessive time and labor costs, and implies the use of toxic and hazardous chemicals. Nowadays, this is undesirable in relation to safety-based reasons and from an economic standpoint. As a consequence, in recent times, the Kjeldahl method is increasingly being replaced by the **Dumas principle**.

Relying on the combustion of the test material and measuring the resulting elemental nitrogen, the **Dumas method** is gaining increasing popularity in food laboratories thanks to its speed and reliability.

Within this scenario, **Proficiency Testing Programs** organized by institutions like **BIPEA** allow the comparison of several laboratories' results, in order to evaluate their analytical performance on the same homogeneous sample applying different methods. By taking part in such programs on a regular basis, the performance of **VELP instruments** is constantly evaluated. Samples provided by BIPEA are analyzed in accordance with the official reference and the obtained results are compared with the given tolerance range.

The **results reported and discussed** in this document are indeed obtained on various feed samples provided by BIPEA as part of the dedicated interlaboratory study

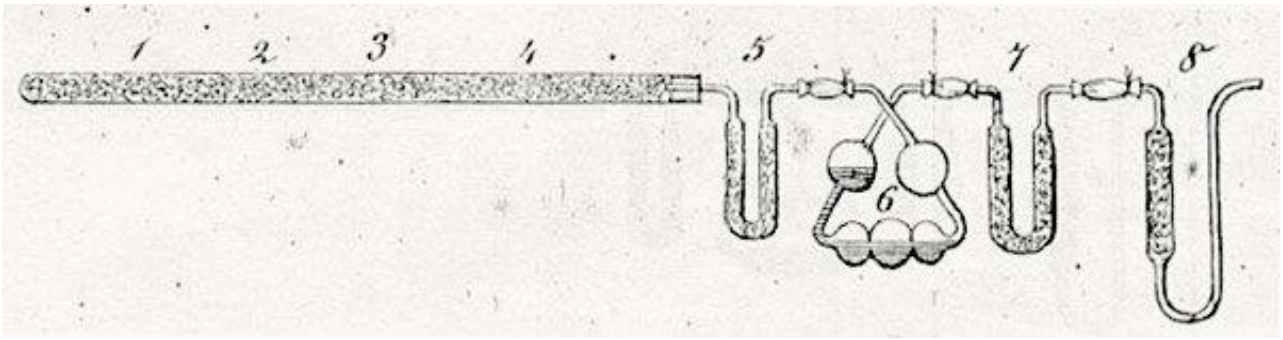
MATERIALS & METHODS

DUMAS AND KJELDAHL METHOD

PROTEIN DETERMINATION

To determine the **quantity of proteins** it is possible to analyze the sample according to the **Dumas method** and the **Kjeldahl method**. Both are primary methods working in accordance with international standards such as AOAC, AACC, ASBC, ISO, IFFO, OIV.

The **Dumas method** for nitrogen determination, developed in 1831, is older than the **Kjeldahl**, 1883, but more convenient in many aspects such as speed, safety, cleanliness, productivity and cost per analysis.



The problem in the past was that it was not easy to reproduce the conditions required by the Dumas method and for this reason, the Kjeldahl technique took the lead and became considered as the classical method for nitrogen/protein determination.

Nowadays, thanks to steps forward in technology, the Dumas nitrogen determination is becoming more widespread.

Results obtained with the Dumas nitrogen determination are usually a little bit **higher than with Kjeldahl** since nitrogen compounds like nitrates, nitrites and heterocyclic compounds are detected.

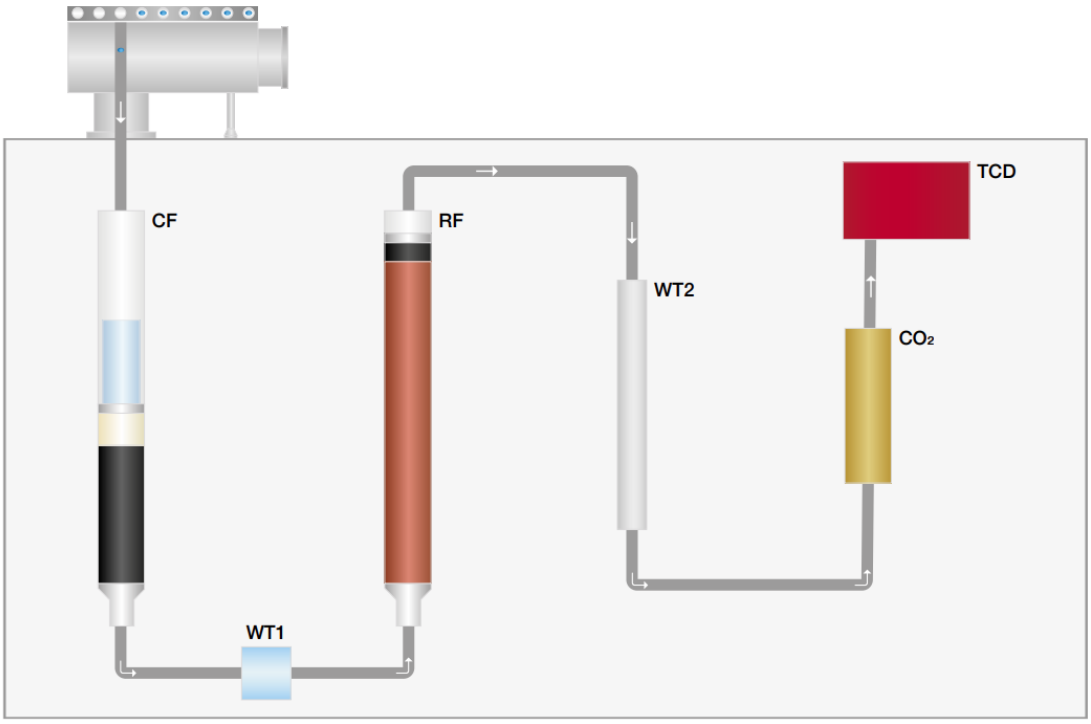
In the **Kjeldahl method**, such compounds are converted into the ammonium ion incompletely or not at all. The opposite could also happen

(rarely) because in this kind of analysis there are lots of variables that could influence the final result.

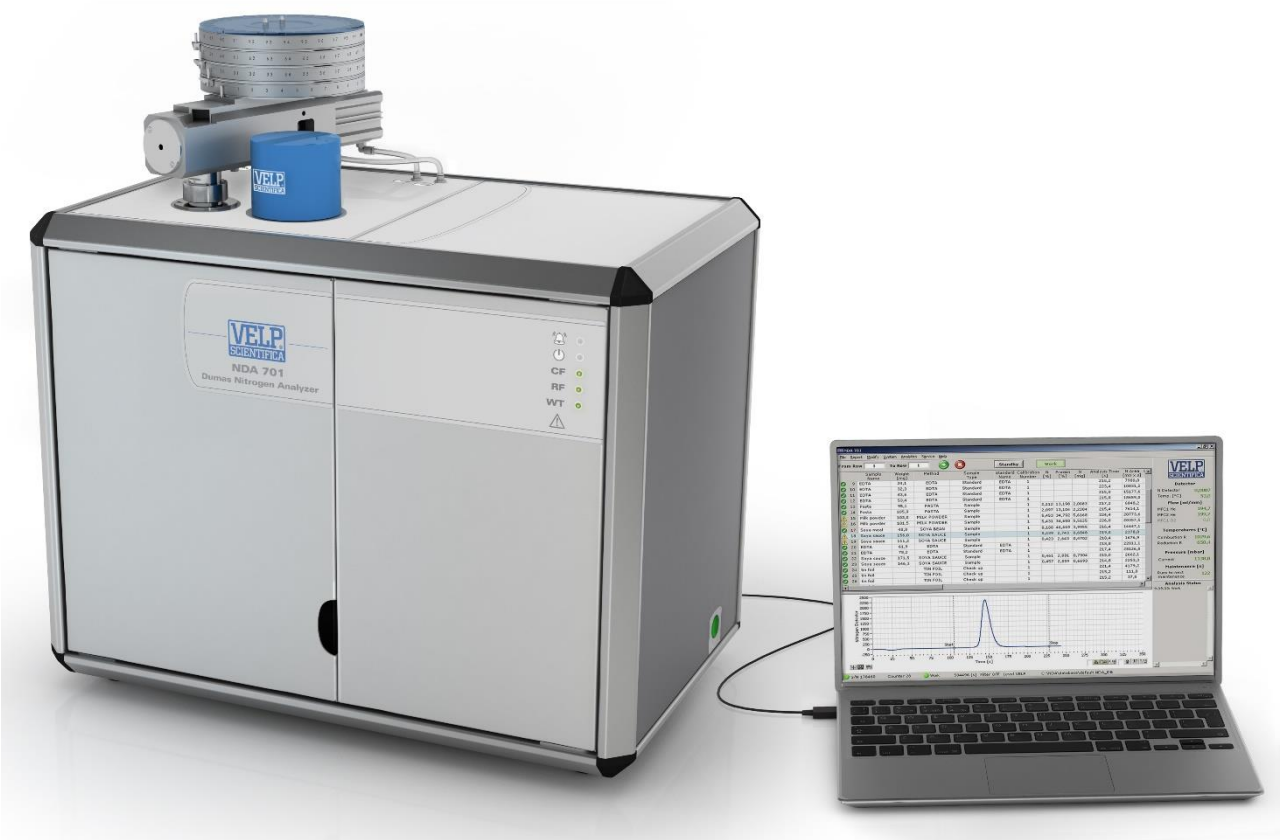
Indeed, there are many minor variants of the Kjeldahl method, involving the use of different catalysts, heating times, volumes and distribution of sulfuric acid and masses of test portion: this shows that the Kjeldahl procedure may be influenced by experimental errors. Recovery is the same for both the methods (≥ 99.5%), while the detection limit is lower for Dumas than for Kjeldahl (0.001 mg N absolute vs. ≥ 0.1 mg N absolute).

DUMAS METHOD

The principle of the Dumas method is to convert nitrogen present in the sample into gaseous NOx by complete combustion in a furnace maintained at 950 - 1.100 °C. The final product (NOx) is then reduced to N_2 and measured using the thermal conductivity detector.



The **Dumas method** starts with a combustion furnace (CF) to burn the sample, obtaining elemental compounds. Water is removed by a first physical trap (WT1) and a second chemical one (WT2). Between the two, the elemental substances pass through a reduction furnace (RF). The auto-regenerative CO2 absorbers let only the elemental nitrogen pass, which is detected by Thermal Conductivity Detector (TCD).



KJELDAHL METHOD

The **Kjeldahl method** consists in a procedure of catalytically supported mineralization of organic material in a boiling mixture of sulfuric acid and sulfate salt at digestion temperatures higher than 400 °C. During the process the organically bonded nitrogen is converted into ammonium sulfate. Alkalizing the digested solution liberates ammonia which is quantitatively steam distilled and determined by titration.

SAMPLE PREPARATION

- Homogenization
- Weighing
- Sulphuric Acid and KjTabs™ Catalyst addition

DIGESTION

- Digestion system configuration with fumes neutralization system and JP Pump
- Method setting (time and temperature ramps)
- Digestion at 420 °C
- Cooling

DISTILLATION

- Vreceiver™ boric acid preparation and addition to the receiving flask
- Tube positioning with the digested sample
- Distiller method setting
- Addition of H_2O - NaOH - Vreceiver™ (different automation level available)
- Removal of residues (different automation level available)

TITRATION

- Manual or automatic
- Colorimetric titration according to AOAC included in automatic distillation units
- External potentiometric titration available for selected models (electrode requires periodical calibration)

METHOD COMPARISON

The main **advantages of the Dumas method** are its ease of use, speed, safety and high level of automation that guarantees unattended operations in the laboratory. The **Dumas method** takes only a few minutes per measurement, making it considerably faster than Kjeldahl analysis, which usually takes more than an hour. Moreover, avoiding the use of toxic and potentially harmful chemicals or catalysts, the Dumas method ensures complete safety for lab operators.

On the other hand, the **Kjeldahl method** uses concentrated sulfuric acid and a catalyst for the digestion of the sample.

As stringent standards started being applied in 1990's with regards to the use of harmful chemicals, many laboratories evaluated the Dumas method as alternative and numerous comparative studies were developed and published. A series of international standards were devised and also grain

inspection services in the US, Canada and Australia recognized 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
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
AOCS Ba 4f-00	Combustion Method for Determination of Crude Protein in Soybean Meal

Key **advantages of the Dumas combustion method** include:

- **Ease of operation** – With the Dumas method you just need to close the sample directly in a tin foil and load it into the elemental analyzer
- **Operator safety** – Dumas' method avoids the use of dangerous chemicals, needed for Kjeldahl method
- **Reduced time of analysis** – starting from 2 hours with Kjeldahl. From 4 minutes per sample with Dumas.
- **No waste production**

MATERIALS

- **NDA 702 – N/Protein Elemental Analyzer**
- **DKL 20 – Digestion Unit**
- **JP Pump and SMS Scrubber for fumes neutralization**
- **UDK 159 – Distillation Unit**

NDA 702 - F30800080

UDK 159 - F30200150

DKL 20 - S30100210

JP Pump - F30620198
SMS Scrubber - F307C0199

CONCLUSIONS

RESULTS & DISCUSSION

PROTEIN ANALYSIS

Table 1 – Kjeldahl and Dumas Protein results

Sample	Method	VELP Results (% P)	Assigned value (% P)	Minimum and Maximum value	BIPEA compliance
15-1213 Soya meal	**Kjeldahl**	**44.5**	45.0	43.6-46.4	**Satisfactory**
	Dumas	**46.1**	45.6	44.2-47.0	**Satisfactory**
8-2913 Wheat draff	**Kjeldahl**	**29.2**	29.5	28.6-30.4	**Satisfactory**
	Dumas	**30.2**	29.9	29.0-30.8	**Satisfactory**
4-1813 Bran	**Kjeldahl**	**15.9**	16.0	15.5-16.5	**Satisfactory**
	Dumas	**16.2**	16.3	15.8-16.8	**Satisfactory**
16-0513 Corn	**Kjeldahl**	**6.4**	6.5	6.1-6.9	**Satisfactory**
	Dumas	**6.6**	6.5	6.1-6.9	**Satisfactory**
11-0313 Fish Meal	**Kjeldahl**	**68.7**	69.0	66.9-71.1	**Satisfactory**
	Dumas	**71.3**	70.1	68.0-72.2	**Satisfactory**
17-2413 Laying hen feed	**Kjeldahl**	**16.1**	16.5	16.0-17.0	**Satisfactory**
	Dumas	**17.0**	16.8	16.3-17.3	**Satisfactory**
8-2513 Poultry meal	**Kjeldahl**	**65.4**	65.4	63.4-67.4	**Satisfactory**
	Dumas	**67.0**	66.4	64.4-68.4	**Satisfactory**

The reported results on feed samples were obtained in accordance with Official Reference Methods, recognized by International Organizations.

Kjeldahl references:

AOAC 2001.11 Protein (Crude) in Animal Feed, Forage (Plant Tissue), Grain, and Oilseeds;

AOAC 984.13 Protein (Crude) in Animal Feed and Pet Food

REG CE 152/2009

EN ISO 5983-2:2009 Animal feeding stuffs

Dumas references:

AOAC 992.23 Crude Protein in Cereal Grains and Oilseeds,

AOAC 990.03 Protein (Crude) in Animal Feed Combustion Method

ISO 16634-1:2008 Oilseeds and animal feeding stuffs

The obtained results for every sample of feedstuff fell within the expected **value range indicated by BIPEA**, demonstrating the high performances of both **VELP Kjeldahl analytical instruments** and **VELP N/Protein Elemental Analyzers**, for **protein determination**.

Both techniques are reliable, efficient and capable of analyzing various feed samples with **high accuracy and repeatability**.

A number of studies suggest that the **Dumas method usually provides a result higher than the Kjeldahl method**. The slight difference is probably due to the near-complete conversion of non-protein forms of nitrogen into elemental nitrogen in the Dumas method, while in the Kjeldahl method nitrates, nitrites and some nitrogenous compounds are converted into the ammonium ion incompletely or not at all.

This has not been seen as a problem, as in **crude protein determination** 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 upon. When using the same conversion factors for techniques with different nitrogen recoveries, differences in results may occur.

VELP UDK 159 – Colorimetric Titration Stages.

VELP N/Protein Elemental Analyzers with high productivity and non-stop performances, are indeed ideal for high throughput, being fully automated and requiring just 3-4 minutes per analysis.

VELP Kjeldahl system, is still a robust solution for protein determination in food and feed field.

It is a great advantage that a single company as **VELP Scientifica**, is able to design, produce and support these two series of instruments to determine the protein content in various feed samples.

Furthermore, **VELP Scientifica internally produces the main consumables** to offer the most suitable **turnkey solution** for your analyses guaranteeing a considerable advantage over the competition, since all the instruments and consumables to optimize the performance of the laboratory come from a single source.

VELP SOLUTIONS

ELEMENTAL ANALYZERS
KJELDAHL ANALYZERS

VELP ELEMENTAL ANALYZERS

NDA 701 – Dumas Elemental Analyzer

- Nitrogen and Protein determination in 3-4 minutes
- Accurate and precise
- Versatile and cloud-enabled
- Helium as carrier gas

NDA 702 – Dumas Elemental Analyzer

- Nitrogen and Protein determination in 3-4 minutes
- Accurate and precise
- Unmatched LOD of 0.001 mgN (with He as carrier gas)
- Versatile and cloud-enabled
- Helium and Argon as carrier gas

CN 802 – Elemental Analyzer

- Carbon, Nitrogen, C:N ratio, TOC and TIC determination
- Fast analysis in 3-5 minutes
- Versatile and cloud-enabled
- Helium and Argon as carrier gas

VELP KJELDAHL ANALYZERS

UDK Series – Distillation Units

- Full range of distillers with different automation to match any lab requirement
- Exclusive patented titanium condenser and steam generator to maximize accuracy and efficiency
- Robust and chemical resistant

DKL Series – Automatic Kjeldahl Digesters

- Fully automatic digesters in 8, 12, 20 and 42 positions
- Stable and homogeneous temperature (±0.5 °C)
- Fast, flexible and safe
- Robust and compact

DK Series – Kjeldahl Digesters

- Semi-automatic digester in 6, 8, 18, 20 and 42 positions
- Stable and homogeneous temperature (±0.5 °C)
- Intuitive and easy-to-use

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